National Library of Canada
Canadian Theses Service

Ottawa, Canada
K1A 0N4

Bibliothèque nationale du Canada
Service des thèses canadiennes

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

NL-339 (r. 88/04) c

Canada

UNIVERSITY OF ALBERTA

Biosynthetic Studies on the Polyketides, Fungichromin and Dehydrocurvularin: Incorporation of Advanced Precursors

by

Zhe Li ©

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Department of Chemistry

EDMONTON, ALBERTA

Spring 1992

National Library
of Canada

Bibliothèque nationale
du Canada

Canadian Theses Service

Service des thèses canadiennes

Ottawa, Canada
K1A 0N4

The author has granted an irrevocable non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of his/her thesis by any means and in any form or format, making this thesis available to interested persons.

The author retains ownership of the copyright in his/her thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without his/her permission.

L'auteur a accordé une licence irrévocable et non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de sa thèse de quelque manière et sous quelque forme que ce soit pour mettre des exemplaires de cette thèse à la disposition des personnes intéressées.

L'auteur conserve la propriété du droit d'auteur qui protège sa thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

ISBN 0-315-73098-6

Canada

UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR Zhe Li

TITLE OF THESIS Biosynthetic Studies on the Polyketides, Fungichromin and Dehydrocurvularin: Incorporation of Advanced Precursors

DEGREE FOR WHICH THESIS WAS PRESENTED Doctor of Philosophy

YEAR THIS DEGREE GRANTED Spring 1992

Permission is hereby granted to THE UNIVERSITY OF ALBERTA LIBRARY to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves other publication rights and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

(SIGNED) ....

PERMANENT ADDRESS:
#144, Seven Persons Cres.,
S.W., Medicine Hat, Alberta
Canada T1B 2A6

DATED January 31, 1992

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Biosynthetic Studies on the Polyketides, Fungichromin and Dehydrocurvularin: Incorporation of Advanced Precursors submitted by Zhe Li in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

....

Dr. John C. Vederas (Supervisor)

....

Dr. William A. Ayer

....

Dr. Ole Hindsgaul

George Kotovych

....

Dr. George Kotovych

....

Dr. Frank Pasutto

Frederick G West

....

Dr. Frederick G. West (External)

Date: January 27, 1992

To Joey

Abstract

The biosyntheses of two polyketide metabolites, fungichromin (**2**) from *Streptomyces cellulosae* ATCC 12625 and dehydrocurvularin (**3**) from *Alternaria cinerariae* ATCC 11784, were investigated by incorporation of advanced precursors.

Ethyl (*Z*)-16-phenylhexadec-9-enoate (**6**), an analogue of ethyl oleate (**5**), was synthesized and administered to the cultures of *S. cellulosae* which normally produce fungichromin (**2**) as the principal polyene antibiotic. These cultures showed reduction of fungichromin biosynthesis but afforded four new polyene antibiotics with truncated four carbon side chains, designated as isochainin (**19**) (an isomer of chainin (**18**)), 14-hydroxyisochainin (**20**), 1'-hydroxyisochainin (**21**), and 1',14-dihydroxyisochainin (**22**). The close correspondence of ^{13}C NMR chemical shifts between these compounds and fungichromin suggests that the stereochemistry at every site is exactly analogous. When two oxaoleate analogues, ethyl (*Z*)-13-butoxytridec-9-enoate (**7**) and ethyl (*Z*)-16-methoxyhexadec-9-enoate (**8**) were synthesized and added to cultures of *S. cellulosae*, polyene production was drastically reduced and no new polyene was detected.

Transformation of ^{14}C-labeled filipin III (**4a**), which has one oxygen atom less than fungichromin (**2**), into fungichromin suggests that **2** is biosynthetically derived from **4a** by insertion of an oxygen atom at the C-14 position of **4a**.

Biosynthesis of the polyketide dehydrocurvularin (**3**) by *A. cinerariae* was examined by incorporation of *N*-acetylcysteamine (NAC) thioesters of (3*S*)-[2,3-$^{13}C_2$]-3-hydroxybutyrate (**34d**), (7*S*,2*E*)-[2,3-$^{13}C_2$]-7-hydroxyoct-2-enoate (**82d**), and (7*S*,2*E*)-[6,7-$^{13}C_2$, 7-*hydroxy*-^{18}O]-7-hydroxyoct-2-enoate (**82f**), in conjunction with potential β-oxidation inhibitors, into **3**. These studies showed that the time of addition of these compounds to cultures of *A. cinerariae* was critical for their intact utilization. The use of β-oxidation inhibitors together with these compounds, as well as a high glucose

replacement medium for the culture, is also crucial for the intact incorporation of the precursors.

Analyses of coupled resonances in the ^{13}C NMR spectra of **3** indicate that the incorporation of **82d** and **82f**, in the presence of 3-tetradecylthiopropanoic acid (**86b**) as a β-oxidation inhibitor, proceeds with very little, if any, degradation of the tetraketide (C_8) portion of the molecule. These results suggest that the enzyme-bound intermediates resembling **34d** and **82d** are the biosynthetic precursors of **3**.

Acknowledgements

I would like to thank my supervisor, Professor John C. Vederas, for his guidance and encouragement during my studies. I would also like to thank Dr. Paul. H. Harrison, Dr. Bernard J. Rawlings, Dr. Yuko Yoshizawa, Dr. Paul B. Reese, and Dr. Fionna M. Martin for their contribution to this thesis. I am grateful to Dr. T. Mark Zabriskie and Dr. Chris Lowe for their help and proof-reading of this manuscript. Professor Michael A. Pickard (Department of Microbiology, University of Alberta) is gratefully acknowledged for his help in the testing of new antibiotics and his collaborative work in the mutagenesis study. Professor E. A. Kean (University of the West Indies, Jamaica) is gratefully acknowledged for a gift of authentic hypoglycin. The assistance of Dr. T. Mark Zabriskie and Dr. Thomas Henkel in running NMR spectra is also appreciated. My thanks are extended to the staff of spectral and analytical services for their assistance in characterizing compounds.

The Natural Sciences and Engineering Research Council of Canada, and the University of Alberta are gratefully acknowledged for financial support .

Table of Contents

Chapter Page

I Introduction 1

Mechanism of Polyketide Formation 1

Fungichromin, a Polyene Antibiotic 7

Dehydrocurvularin, a Macrocyclic Phytotoxin 11

II Results and Discussion 15

Biosynthetic Studies on Fungichromin 15

Syntheses of Oleate Analogues and Production of New Polyene Antibiotics 15

Studies on the Biosynthetic Relationship of Fungichromin and Filipins 27

Syntheses and Incorporation of Advanced Precursors into Fungichromin 30

Biosynthetic Studies on Dehydrocurvularin and Incorporation of Advanced Precursors 38

Introduction: Intact Incorporation of Advanced Polyketide Intermediates 38

The β-Oxidation Pathway 40

Introduction to Inhibition of β-Oxidation 43

Intact Incorporation of Advanced Precursors into Dehydrocurvularin 49

Conclusions 64

Experimental 66

References 136

List of Tables

Table Page

1 ^{13}C Chemical shifts for fungichromin (**2**), isochainin (**19**), 14-hydroxyisochainin (**20**), 1'-hydroxyisochainin (**21**), and 1',14-dihydroxyisochainin (**22**) 19-20

2 Effect of β-oxidation inhibitors on intact incorporation of **34d** into dehydrocurvularin (**3**) 52

3 Effect of β-oxidation inhibitors on intact incorporation of **82d** into dehydrocurvularin (**3**) 60

4 ^{13}C Chemical shifts for fungichromin (**2**), filipin III (**4a**), and filipin II (**4b**) 71-72

List of Figures

Figure		Page
1	Structures of fungichromin (**2**) and dehydrocurvularin (**3**)	7
2	Structures of filipins (**4**)	8
3	Structures of some polyene antibiotics	9
4	Structures of curvularin (**1**) and dehydrocurvularin (**3**)	11
5	Structures of curvularin-type metabolites	14
6	Structures of isochainin (**19**), 14-hydroxyisochainin (**20**), 1'-hydroxyisochainin (**21**), 1',14-dihydroxyisochainin (**22**) and pentamycin (**23**)	21
7	The production of polyenes with Span 85	23
8	The production of polyenes with ethyl oleate (**5**)	24
9	Structures of some β-oxidation inhibitors	43
10	Expansions of ^{1}H-decoupled ^{13}C NMR spectra of **3** after incorporation of **34d**	51
11	Expansions of ^{1}H NMR spectra of **41a** after addition of optically active NMR shift reagent Eu(hfc)$_3$	57
12	Expansions of ^{1}H NMR spectra of **51** after addition of optically active NMR shift reagent Eu(hfc)$_3$	58
13	Expansions of ^{1}H-decoupled ^{13}C NMR spectra of **3** after incorporation of **82d**	60
14	Expansions of ^{1}H-decoupled ^{13}C NMR spectra of **3** after incorporation of **82f**	64

List of Abbreviations

Ac	acetyl
Bu	butyl
n-BuLi	*n*-butyl lithium
CI	chemical ionization
CoA	coenzyme A
DCC	1,3-dicyclohexylcarbodiimide
DIBAL	diisobutylaluminum hydride
DMAP	4-dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DMSO	dimethylsulfoxide
DPM	decompositions per minute
DPPA	diphenylphosphoryl azide
EI	electron impact ionization
Enz	enzyme
Et	ethyl
FAB	fast atom bombardment
FID	flame ionization detector
GC	gas chromatography
HPLC	high performance liquid chromatography
IR	infrared spectroscopy
LiHMDS	lithium hexamethyldisilazide
MCPA	methylenecyclopropaneacetic acid
Me	methyl
MPLC	medium pressure liquid chromatography

MS	mass spectrometry
NAC	*N*-acetylcysteamine
NMR	nuclear magnetic resonance
PCC	pyridinium chlorochromate
Ph	phenyl
PPL	porcine pancreatic lipase
Pr	propyl
Py	pyridine
R_t	retention time
TBDMS	*tert*-butyldimethylsilyl
THF	tetrahydrofuran
THP	tetrahydropyranyl
TLC	thin layer chromatography
TMS	tetramethylsilane
Ts	*p*-toluenesulfonyl

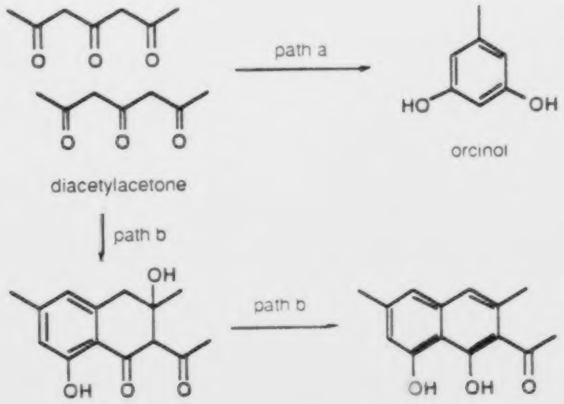
INTRODUCTION

MECHANISM OF POLYKETIDE FORMATION

The polyketides are members of a large and diverse class of natural products that includes substances such as phenols, quinones, xanthones, flavonoids, and numerous mycotoxins. These compounds are generally secondary metabolites of bacteria, fungi, or plants. Many of them exhibit interesting biological properties. Typical examples of polyketides exhibiting such properties are: daunorubicin - an anticancer agent; erythromycin, oleandomycin, leucomycin, spiramycin and midecamycin - clinically useful antibiotics; FK 506 - an immunosuppressant; aflatoxins and ochratoxins - potent carcinogenic agents.

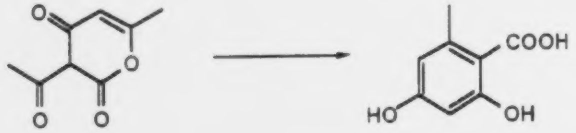
The diversity of polyketide structure is bound to a common origin; their molecular architecture is constructed according to a regular structural pattern. Collie (1907) found that many "polyacetates" could undergo basic cyclization reactions to produce phenolic compounds.[1] For example, when diacetylacetone (a triacetic acid) is treated with strong alkali, orcinol forms through intramolecular condensation (Scheme 1, path a). Under weakly alkaline conditions, intermolecular condensation predominates (Scheme 1, path b).

Scheme 1.



Similarly, dehydroacetic acid, a tetraketide, rearranges to orsellinic acid under basic conditions (Scheme 2). Collie suggested these and related experimental observations could account for the biological formation of many natural products, but his theory was ignored.

Scheme 2.

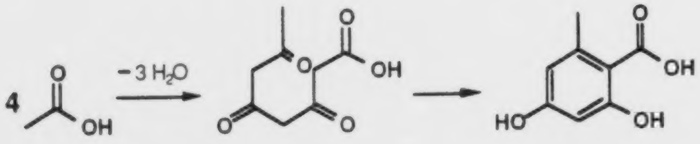


dehydroacetic acid

orsellinic acid

In the early 1950s, Birch suggested that in their biosyntheses, polyketides are derived from β-polyketomethylene (or polyacetate), which itself originated from head-to-tail linkage of acetates by a process similar to fatty acid formation.[2] The "acetate hypothesis" came largely from attempts to extrapolate the known biological significance of acetic acid as a building unit in the biosyntheses of fatty acids and sterols, to phenolic and enolic compounds. In the original hypothesis, a β-polyketomethylene intermediate could then undergo secondary reactions, such as aldol or Claisen cyclizations, to form the ring structure. Subsequent modifications of the molecule by oxidation, dehydration, or alkylation would then afford the final polyketide product. An example of the application of this hypothesis to the biosynthesis of orsellinic acid is shown in Scheme 3.

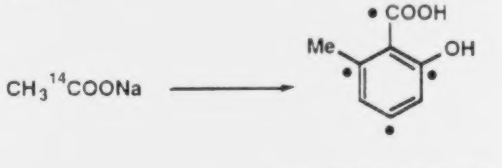
Scheme 3.



polyketide intermediate

orsellinic acid

In 1955, Birch and coworkers utilized *in vivo* incorporation of radiolabeled acetate into specific locations in 6-methylsalicylic acid to provide the first experimental evidence for the "acetate hypothesis".[3]

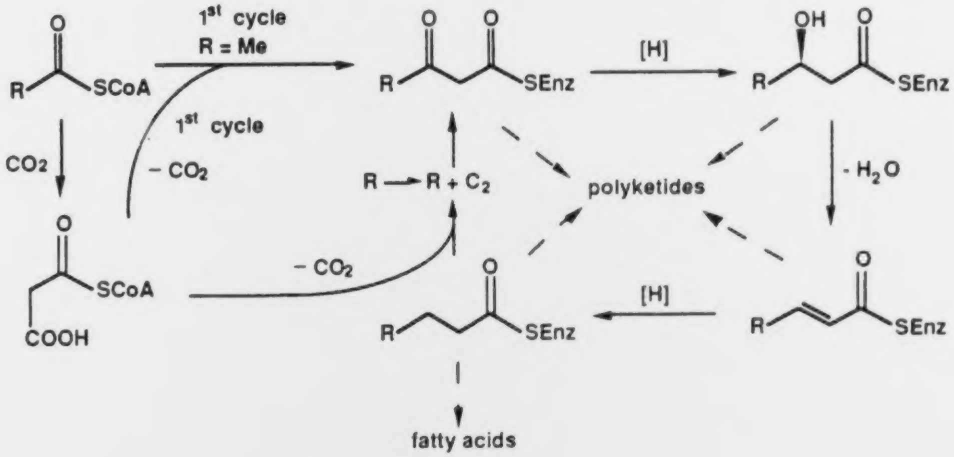

$CH_3{}^{14}COONa$
• COOH
Me
OH

6-methylsalicylic acid

Since then, this idea has been supported by many experiments. Radioactive labeling in the biosynthetic field, and the use of stable isotopes in combination with modern NMR techniques have become powerful and standard operations for the elucidation of biosynthetic pathways and have assisted in structure determination.[4]

It is now generally accepted that polyketide biosynthesis occurs through a series of condensations of two carbon units in a manner similar to that of formation of fatty acids as proposed by Lynen.[5] Fatty acid synthase proceeds by condensation of a starter unit (commonly acetate) to an extender unit (malonate) with concomitant decarboxylation (Scheme 4). The β-keto group of the resulting extended chain is then fully processed

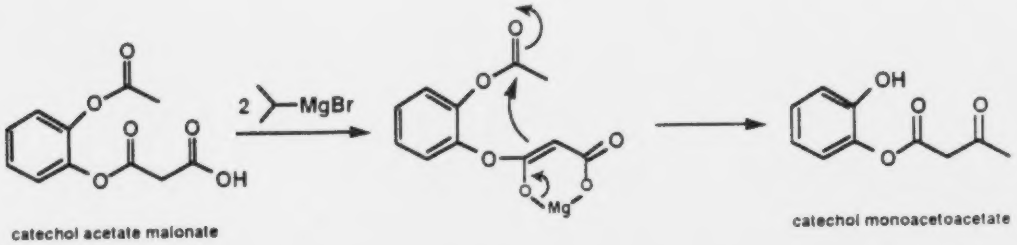
Scheme 4.


R
SCoA
1st cycle
R = Me
1st cycle
− CO2
CO2
SCoA
COOH
− CO2
R ⟶ R + C2
SEnz
[H]
OH
SEnz
polyketides
- H2O
[H]
SEnz
SEnz
fatty acids

(reduced), and the cycle resumes with the condensation of a new extender unit. However, most polyketides contain structural complexities. These arise principally by the use of different extender units at certain steps and variations in the extent of processing of the β-carbon (β-keto reduction, dehydration, reduction) as shown in Scheme 4.

Two models for the first steps in the biosynthesis of polyketides have been reported.[6,7] Scott and co-workers used an acylated catechol model to mimic the condensing enzyme complex in polyketide biosynthesis.[6] Catechol acetate malonate, upon treatment with two equivalents of isopropylmagnesium bromide, undergoes an intramolecular acetyl transfer reaction to form catechol monoacetoacetate (a chain elongation product) (Scheme 5).

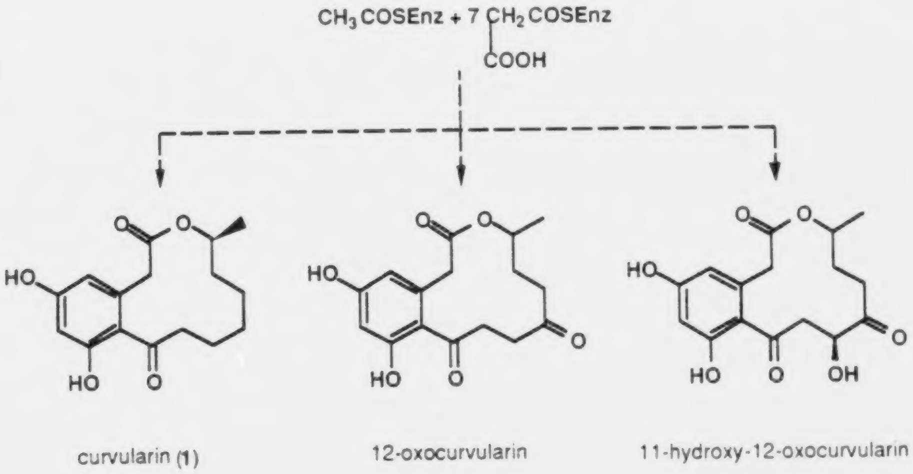
Scheme 5.



In a second model, a similar intermolecular acetyl transfer reaction was successfully achieved by using a thiolmalonate-thiolacetate system.[7]

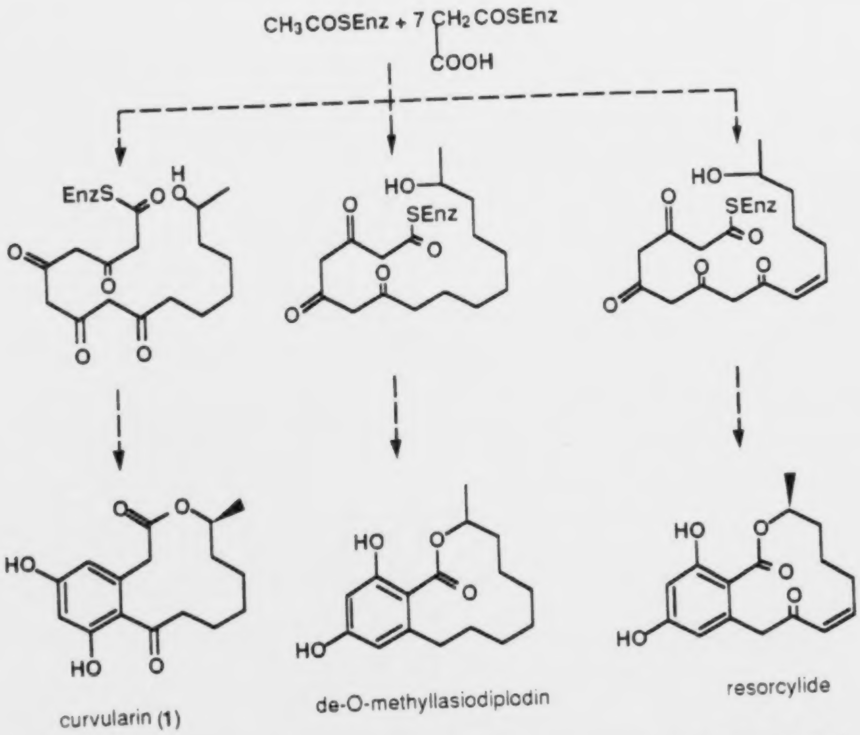
The nature of the polyketide synthases, which appear to be multienzyme complexes that resemble the fatty acid synthases, still remains unclear. With the exception of polyketide synthases that form simple aromatic compounds (e.g. 6-methylsalicylic acid synthase, orsellinate synthase, and chalcone synthases),[8] the cell free production of complex polyketides or isolation of their assembly enzymes has not been reported. The condensation process of simple building blocks is very complex and includes a whole array of consecutive reactions which are still rather poorly understood. For example, in the biosyntheses of curvularin (**1**) and curvularin-related compounds (Scheme 6 and 7),[9-11] the metabolites may result from different degrees of processing (e.g., reduction and dehydration) (Scheme 6)[11] and different folding (Scheme 7)[9,10] of the polyketide intermediates.

Scheme 6.



CH3COSEnz + 7 CH2COSEnz
COOH
HO
HO
O
O
O
OH
curvularin (1)
12-oxocurvularin
11-hydroxy-12-oxocurvularin

Scheme 7.

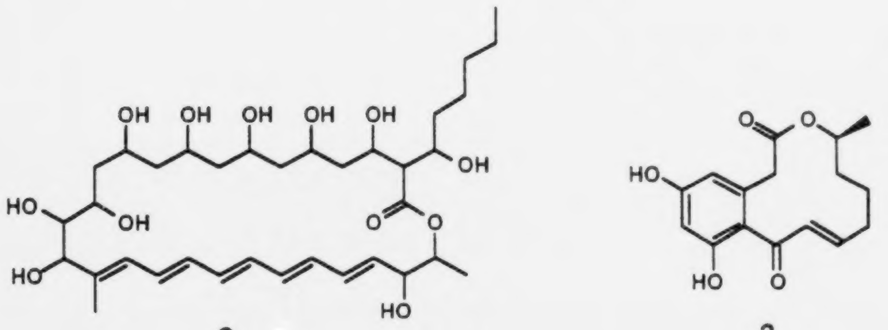


Recently, some progress has been made regarding the roles of hypothetical intermediates such as *propionate*-derived di- and triketides in the biosyntheses of erythromycin,[12] tylactone,[13] nargenicin,[12b,14] and nonactin.[15] In these investigations, ^{13}C labeled di- and triketides, activated as the *N*-acetylcysteamine (NAC) thioesters were fed to producing cultures of the relevant microorganisms. A small portion of these key precursors was incorporated intact into the antibiotics. Several branched chain fatty acids and related ketones[16-20] which represent putative intermediates in the formation of the parent aglycones for the sixteen-membered ring macrolides, tylosin[16] and mycinamicin,[17] were isolated both from mutants and producing cultures of *Streptomyces* and *Micromonospora* species.

Genetic research has provided key insights into the mechanism of polyketide biosynthesis.[21-28] The sequence of the *eryA* gene of *Saccharopolyspora erythraea* which encodes the presumptive polyketide synthase responsible for the formation of the erythromycin aglycone, 6-deoxyerythronolide B, was independently reported by two groups.[21,22] These genes are organized in six repeated units that encode fatty acid synthase (FAS)-like activities. Each of these genes appears to encode a functional unit which is responsible for one of the six chain elongation steps required for the formation of this polyketide.

The work cited above centers on the biosyntheses of *propionate-derived* polyketides. However, intact utilization of functionalized *acetate-derived* polyketides has not been previously reported. Thus, we wish to incorporate advanced precursors into *acetate-derived* polyketide antibiotics such as fungichromin (**2**) and dehydrocurvularin (**3**) (Figure 1) and therefore to determine their biosynthetic pathway. A part of this work recently has been published.[29]

Figure 1. Structures of fungichromin (**2**) and dehydrocurvularin (**3**)



2

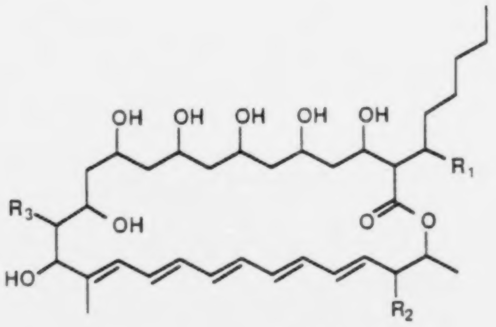
3

FUNGICHROMIN, A POLYENE ANTIBIOTIC

Fungichromin (**2**) and the filipins (**4**) (Figure 2) belong to the group of macrocyclic polyene antibiotics, a class of over 200 compounds, produced by *Streptomyces* species.

that possess antifungal and antiprotozoal activity.[30,31]

Figure 2. Structures of filipins (**4**)



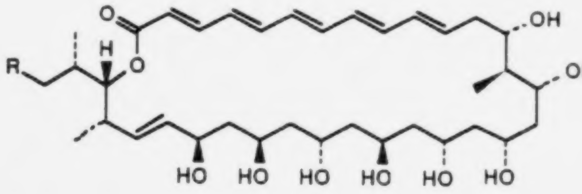
fungichromin 2 $R_1 = R_2 = R_3 = OH$
filipin III 4a $R_1 = R_2 = OH$, $R_3 = H$
filipin II 4b $R_1 = R_2 = H$, $R_3 = OH$
filipin I 4c $R_1 = R_2 = R_3 = H$

All the polyene antibiotics have certain common structural features. They contain a macrolide lactone ring ranging from 14 to 44 atoms. The presence of the lactone confers a highly characteristic peak on the infrared spectra of these compounds. This ring has a set of three to eight conjugated double bonds on one side and a set of hydroxyl groups on the other side. The polyenes absorb very strongly in the ultraviolet, with the absorption maxima depending on the length of the polyene chain. Accordingly, they can be classified as tetraenes, pentaenes, hexaenes, or heptaenes by the characteristic UV-visible absorptions of their chromophore.[32-37]

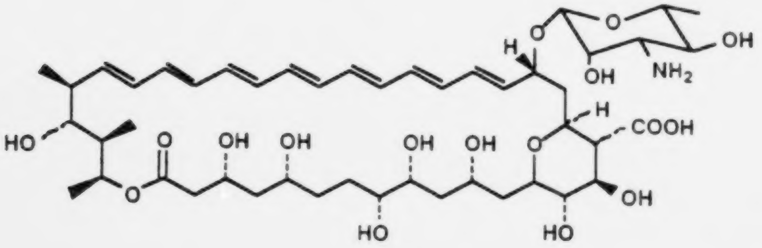
Several members of this family have been in world-wide clinical use for many years despite their toxicity.[38] For example, amphotericin B and nystatin (Figure 3) remain the best treatment for many fungal infections in humans, and some polyenes also act synergistically with antitumor agents.[39] Their activity is due to their ability to interact with sterols in cytoplasmic membranes to generate pores which allow loss of cellular constituents.[38]

The isolation of pure polyene compounds is often difficult because they are initially present at very low concentrations, and in many cases, several structurally similar polyenes co-occur. They also display limited solubility in both aqueous solution and organic solvents and tend to be non-crystalline and highly reactive. Despite the long medical use of these polyene antibiotics, until recently the only member of this class whose complete stereochemical structure had been determined was amphotericin B (Figure 3).[40] The lack of stereochemical information is an obstacle to the study of structure-activity relationships, which would assist development of therapeutically useful compounds.

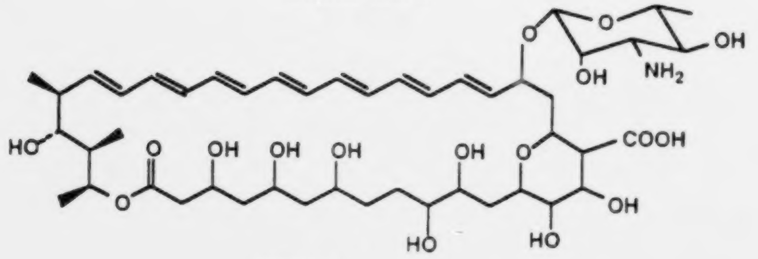
Figure 3. Structures of some polyene antibiotics


O
R
H
O
OH
OH
HO HO HO HO HO HO

mycoticin A R = H
mycoticin B R = Me


H
O
O
OH
NH2
OH
HO
O
OH OH
OH OH
O
H
COOH
OH
HO
HO

amphotericin B


O
O
OH
NH2
OH
HO
O
OH OH OH
OH
O
COOH
OH
HO
HO

nystatin A₁

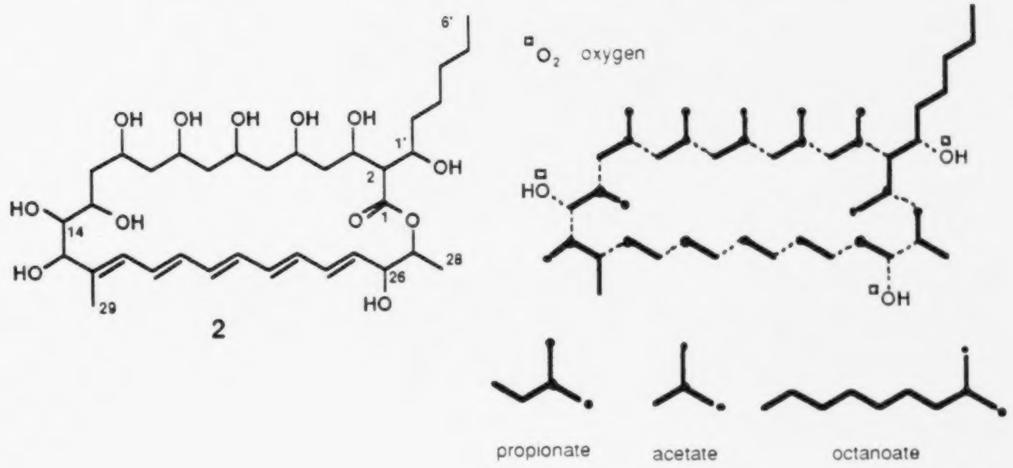
However, great progress has recently been made on chemical synthesis, structure elucidation, stereochemistry, and isolation of new polyene antibiotics.[41-50] For instance, Nicolaou's group accomplished the total synthesis of amphotericin B in 1988.[41a] In recent work, Schreiber *et al.* elucidated the complete stereostructure of mycoticins A and B (Figure 3).[49] NMR techniques have been used for the assignments of the stereostructure of vacidin A,[47b] pimaricin,[48d] and nystatin A[48a] (Figure 3). The stereochemical assignments of pentamycin, a polyene antibiotic from *Streptomyces pentaticus* with the same gross structure as fungichromin (**2**), have also been reported.[50]

Biosynthetically, the polyene antibiotics are typical polyketide metabolites. Hence, the macrocyclic ring of polyenes probably arises from acetate and propionate.[51] Birch *et al.*[52] first found good incorporation of [^{14}C]-labeled acetate and propionate into nystatin aglycone, and similar results have been obtained with amphotericin B,[53] lucensomycin,[54] candicidin,[55] fungimycin,[56] and levorin.[57]

Fungichromin (**2**) is produced by *Streptomyces cellulosae*.[31b,31c] The antibiotic is identical with lagosin[31a] from *Streptomyces roseoluteus* and with cogomycin[31a] from *Streptomyces fradiae*. The structure of **2** has been independently determined by two separate groups.[58] Filipins (**4**), polyketide metabolites[51c] from *Streptomyces filipinensis* having very similar structures,[59] co-occur with fungichromin (**2**) in *S. cellulosae*.

Studies by the Vederas group on fungichromin (**2**) were the first examples of the use of NMR and stable isotope techniques to examine polyene antibiotic biosynthesis.[60] From this work, it is clear that fungichromin (**2**) is derived from one propionate unit, twelve acetate units, and one intact octanoate unit, condensed in the head-to-tail fashion typical of polyketide biogenesis (Scheme 8).[60] Interestingly, the side chain of **2** (C-1 to C-6' fragment) is derived exclusively from oleate, as demonstrated by the incorporation of ethyl [18-$^2H_3$] oleate into **2**.[60]

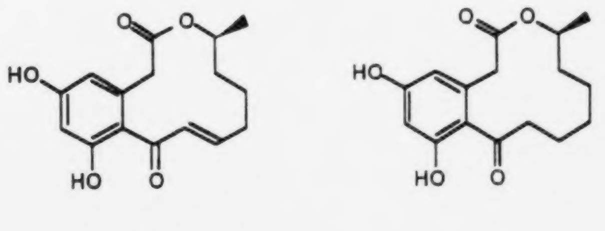
Scheme 8.


6'
OH
OH
OH
OH
OH
1'
OH
2
HO
14
OH
O
1
O
HO
26
28
29
HO
2
O_2 oxygen
HO
OH
OH
propionate
acetate
octanoate

DEHYDROCURVULARIN, A MACROCYCLIC PHYTOTOXIN

The macrocyclic lactone dehydrocurvularin (**3**) and closely related compounds (e.g., curvularin (**1**)) (Figure 4) are produced by a number of fungal species, especially members of the genus *Alternaria* which are potent plant pathogens.[61]

Figure 4. Structures of curvularin (**1**) and dehydrocurvularin (**3**)

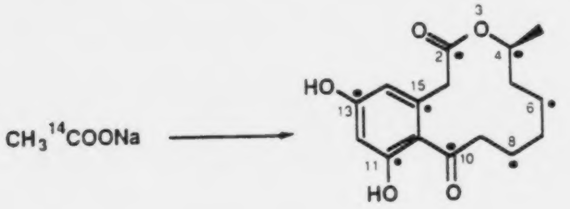

HO
HO
O
O
O
HO
HO
O
O
O

3

1

This class of compounds possesses interesting biological properties.[62] Robeson and co-workers[61i] identified the major phytotoxic component from *Alternaria macrospora* as dehydrocurvularin (**3**); this fungus causes a cotton leaf spot and twig blight disease, and has been found on cotton in China, Africa, India, South America, Israel, and the USA.[63] Since this compound also attacks weeds, it has been suggested as a potential biocontrol agent.[64] Recently, dehydrocurvularin (**3**) and curvularin (**1**) have been reported to have remarkable inhibitory activities against the proliferation of sea urchin embryo cells, and therefore could be used potentially as regulators in studying the mechanisms of cell growth.[65]

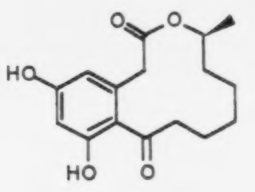
Curvularin (**1**) was isolated from the culture filtrate of a species of *Curvularia* in 1952, and its name was proposed by Musgrave in 1956.[66] Chemical syntheses of **1** have been reported by several groups,[67-70] and its biosynthesis was investigated by Birch and coworkers in 1959.[71] Incorporation of [1-^{14}C]acetic acid into curvularin (**1**), and Kuhn-Roth oxidation gave acetic acid from C-4 and the attached methyl group. Degradation of this acetic acid demonstrated that all of its radioactivity is in the carboxyl carbon.



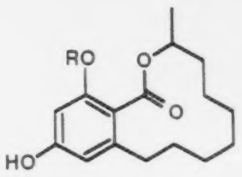
1

A head-to-tail condensation of eight acetic acid units was proposed on the basis of these results.

Secondary metabolites having structural features similar to **1** have also been reported.[9,10] Lasiodiplodin and de-O-methyllasiodiplodin were isolated from *Lasiodiplodia theobromae*.[9] Their structures were determined by chemical degradation and



curvularin (**1**)

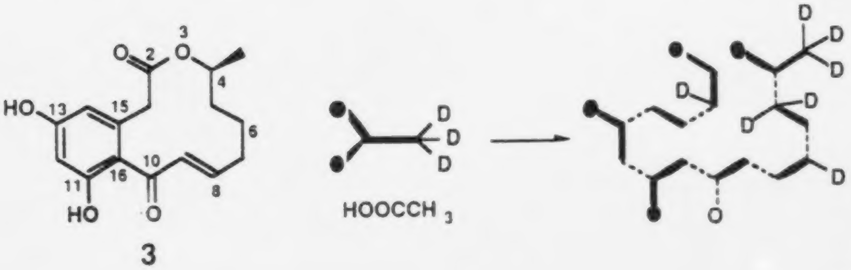


R = Me, lasiodiplodin
R = H, de-O-methyllasiodiplodin

spectrometric methods. The fungal metabolites *cis*-resorcylide and *trans*-resorcylide are potent plant-growth inhibitors isolated from a *Penicillium* species.[10] Two aliphatic 12-membered lactones, recifeiolide[72] and cladospolide A,[73] were also reported.

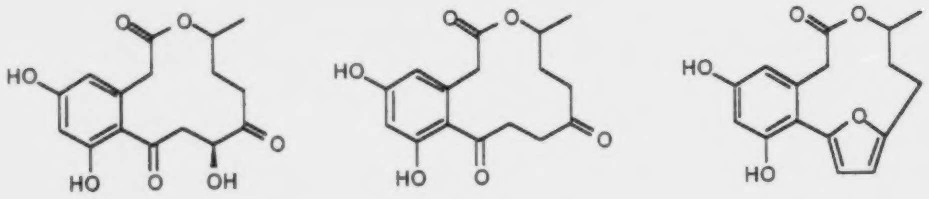
Our group has described the unambiguous NMR assignments and biosynthesis of dehydrocurvularin (**3**).[74] Incorporations of sodium [2-$^2H_3$]acetate and [1-^{13}C, $^{18}O_2$]acetate show that **3** is derived from eight acetate units in head-to-tail fashion, and that a number of intact carbon-deuterium bonds and carbon-oxygen bonds are derived from the labeled acetates (Scheme 9). The acetate-derived deuterium occupies the *pro-S* positions at C-7 of **3**. Interestingly, this stereochemical outcome is opposite to that obtained for fatty acid biosynthesis in the same culture.[74]

Scheme 9.



Very recently, compounds related to **3**, 11-hydroxy-12-oxocurvularin, 12-oxocurvularin, and citreofuran, have been isolated from a hybrid strain, ME 0005, derived from *Penicillium citreo-viride* B IFO 6200 and 4692 (Figure 5).[11] NMR analyses of these metabolites after incorporation of sodium [1,2-$^{13}C_2$]acetate indicate that eight acetates are utilized to construct each of these compounds.

Figure 5. Structures of curvularin-type metabolites



11-hydroxy-12-oxocurvularin

12-oxocurvularin

citreofuran

Fungichromin (**2**) and dehydrocurvularin (**3**) were selected for studies of the mechanism of polyketide formation, not only because both antibiotics have interesting biological properties, but also because of the variety of oxidation states present along their *acetate-derived* polyketide chains. Furthermore, it also appeared possible that new polyene antibiotics could be produced by *S. cellulosae* through incorporation of oleate analogues into the side chain of fungichromin (**2**).

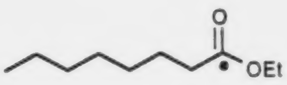
RESULTS AND DISCUSSION

BIOSYNTHETIC STUDIES ON FUNGICHROMIN

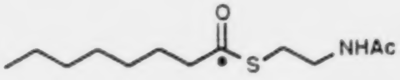
Syntheses of Oleate Analogues and Production of New Polyene Antibiotics

Our previous biosynthetic studies[60] on fungichromin (**2**) indicate that it is a typical polyketide as detailed in Scheme 8. Analysis of the ^{13}C NMR spectra of samples of **2** derived from labeled acetate reveals that, in contrast to the normal 2- to 3-fold signal enhancements observed in the macrocyclic ring of **2**, there are no detectable enrichments in the C-1' to C-6' portion. Indeed, no coupled satellites were observed for any of these signals after incorporation of [1,2-$^{13}C_2$]acetate; this type of experiment has been shown to be more sensitive to small incorporations of labeled precursors.[75,76] Subsequently, sodium [1-^{13}C]octanoate and sodium [3-^{13}C]octanoate were incorporated into fungichromin (**2**) in two separate feeding experiments; the ^{13}C NMR spectra showed C-1 or C-1' to be the sole site of enrichment (ca. 3%), respectively. Feeding sodium [1-^{13}C]hexanoate to cultures of *S. cellulosae*, to test the specificity of octanoate as a unit, gave **2** in which none of the carbon atoms are labeled.

It seemed feasible that incorporation of chemically modified analogues of the octanoate unit could give modified antibiotics. Since the incorporation rate of sodium octanoate into fungichromin (**2**) is low, more efficient precursors were sought. Ethyl [1-^{13}C]octanoate and the *N*-acetylcysteamine (NAC) thioester of [1-^{13}C]octanoate[31] were synthesized.



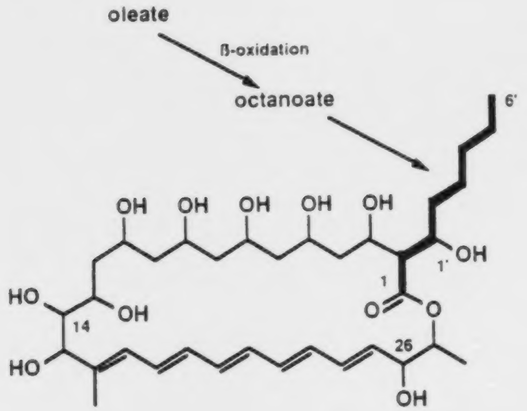
Ethyl [1-^{13}C]octanoate



NAC thioester of [1-^{13}C]octanoate

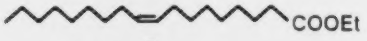
The NAC thioester was chosen because it is thought to mimic the CoA ester and has been used with success in incorporation studies with advanced putative intermediates in polyketide biosynthesis.[12,13] Both precursors were specifically incorporated into **1**, but the levels of enrichment were low, possibly due to rapid hydrolysis of their ester groups by the organism. Further studies by Dr. Paul Harrison found that replacement of the fatty acid source (e.g., Span 85, a mixture whose principal component is sorbitan tri-oleate) in the medium, with a mixture of 10% ethyl [18-$^2H_3$]oleate and ethyl oleate (**5**) gave fungichromin (**2**) in which C-6' was extensively deuterated ^2H_3C, as determined by ^2H NMR. These results, together with the observation that [U-^{13}C]glucose is not incorporated into the octanoate unit of **2**, suggest that oleate acts as the sole carbon source for the C-1 to C-6' portion of **2** (Scheme 10). This may also explain why oleate is required in order to obtain good production of **2**.[77]

Scheme 10.

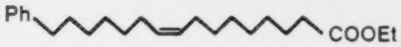


It is known that oleate is catabolized by β-oxidation to form dodec-3-enoate, which is then isomerized to dodec-2-enoate.[78,79] An enzyme-catalyzed 1,4-addition of water to the α,β-unsaturated system then follows to give 3-hydroxydodecanoate. Further β-oxidation of this compound to octanoate in *S. cellulosae*, would afford a plausible explanation for the observed results.

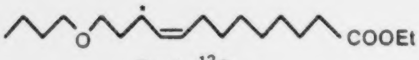
Hence it seemed that replacement of oleate by an analogue in the normal fermentation medium could result in new polyenes with a modified side chain (C-1' to C-6'). A number of oleate analogues were then designed and synthesized, including ethyl (*Z*)-16-phenylhexadec-9-enoate (**6**), ethyl 14-oxaoleate [(*Z*)-13-butoxytridec-9-enoate] (**7**), ethyl 17-oxaoleate [(*Z*)-16-methoxyhexadec-9-enoate] (**8**), and ethyl 18-hydroxyoleate [(*Z*)-18-hydroxyoctodec-9-enoate] (**9**).



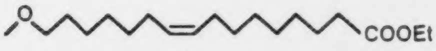
5



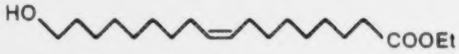
6



7a • = ^{12}C
7b • = ^{13}C / ^{14}C



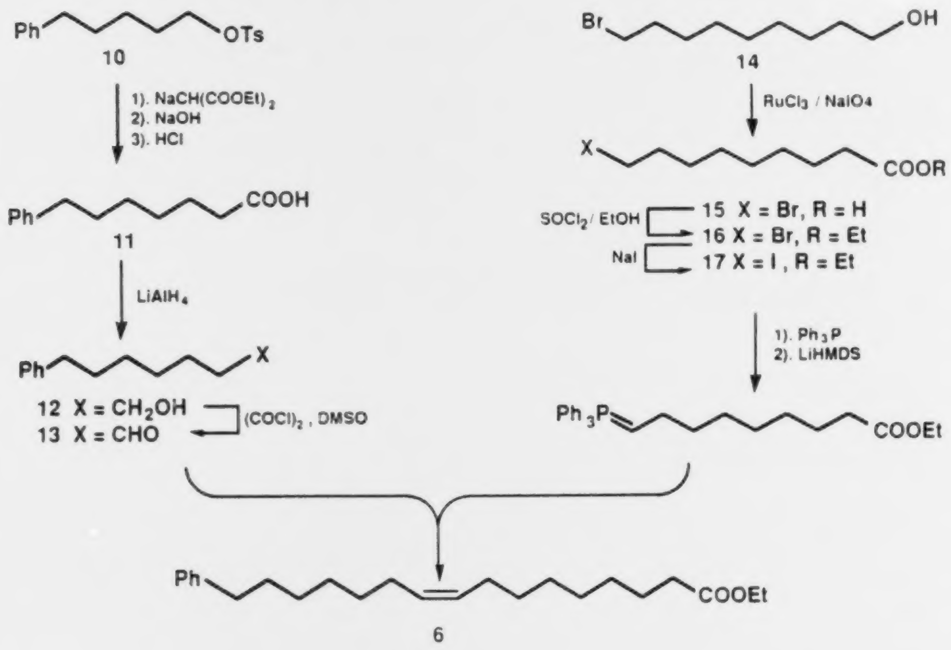
8



9

The oleate analogue **6** was synthesized as depicted in Scheme 11. Commercially available 5-phenylpentanol was transformed to its tosylate **10** and extended by two carbon

Scheme 11.


Ph
OTs
10
1). NaCH(COOEt)$_2$
2). NaOH
3). HCl
Ph
COOH
11
LiAlH$_4$
Ph
X
12 X = CH$_2$OH
13 X = CHO
(COCl)$_2$, DMSO
Br
OH
14
RuCl$_3$ / NaIO$_4$
X
COOR
SOCl$_2$/ EtOH
15 X = Br, R = H
16 X = Br, R = Et
NaI
17 X = I , R = Et
1). Ph$_3$P
2). LiHMDS
Ph$_3$P
COOEt
Ph
COOEt
6

atoms via malonic ester synthesis to give 7-phenylheptanoic acid (**11**), which was converted to 7-phenylheptanal (**13**) by a reduction-oxidation sequence (22% overall yield).

The other half of **6** was prepared from 1,9-nonandiol. Treatment of this compound with 48% HBr in refluxing benzene with the azeotropic removal of water gives 9-bromononanol (**14**) in 65% yield.[80-82] Oxidation of the 9-bromononanol (**14**) with concentrated HNO_3 using a literature procedure afforded a complex mixture.[83] Fortunately, sodium periodate together with a catalytic amount of ruthenium trichloride,[84] easily oxidizes **14** to the corresponding acid **15** in 89% yield. Esterification (98%) with thionyl chloride and ethanol[85] to **16**, and halogen exchange with NaI[86] produced ethyl 9-iodononanoate (**17**) in 97% yield.

The triphenylphosphonium iodide salt, obtained from reaction of **17** with triphenylphosphine, is treated with LiHMDS to give the triphenylphosphonium ylide *in situ*, which condenses with **13** to give a 51% yield of the desired *Z* isomer of ethyl 16-phenylhexadec-9-enoate (**6**).[87] The stereochemistry of the double bond in **6** is known to be *cis* from the 10.5 Hz coupling constant between the olefinic hydrogens. Although these two protons have nearly identical chemical shifts, the coupling can be seen in the ^1H NMR spectrum at the small satellite signals arising from species bearing natural abundance carbon-13 at the olefinic carbons, provided that the allylic hydrogens are simultaneously decoupled by homonuclear irradiation.

Compound **6** was added in varying amounts (0.5 to 5.0 g per liter) to growing cultures of *S. cellulosae* as a replacement for the oleate esters (e.g., Span 85 or **5**) normally used in the medium. Despite reasonably good growth of the organism, production of fungichromin (**2**) was greatly depressed by **6**. However, small quantities of four previously undetected polyene antibiotics could be isolated in pure form by HPLC. Our previous unambiguous assignment[60] of all ^{13}C NMR resonances of fungichromin (**2**) was the key tool for structure elucidation of these compounds. Comparison of the carbon chemical shifts (Table 1) showed very close correspondence except for two or three areas of structural difference. This information together with the positive ion fast atom bombardment mass spectra (POSFAB MS) and UV spectra characteristic of

Table 1. ^{13}C Chemical shifts for fungichromin (**2**), isochainin (**19**), 14-hydroxyisochainin (**20**), 1'-hydroxyisochainin (**21**) and 1',14-dihydroxyisochainin (**22**).

carbon	^{13}C $\delta^{a,b}$	^{13}C δ^{a}			
	2	**19**	**20**	**21**	**22**
29	11.74	11.45	11.80	11.08	11.70
6'	14.38	---	---	---	---
28	17.96	18.29	18.30	17.95	17.91
5'	23.65	---	---	---	---
3'	26.01	23.60	23.61	19.51	19.52
4'	32.88	14.21	14.25	14.23	14.23
2'	36.22	29.87	30.18	38.36	38.40
12	39.58	42.52	39.50	41.58	39.54
4	41.38	42.70	42.33	42.86	41.34
10	44.34	44.20	44.15	44.18	44.36
6	45.17	44.91	44.83	45.17	45.21
8	45.33	45.11	45.16	45.26	45.36
2	60.35	54.26	54.40	60.31	60.46
13	70.34	67.50	70.26	67.47	70.38
11	71.45	71.00	71.35	71.12	71.46
1'	72.59	30.60	30.57	72.28	72.21
26	73.25	73.15	73.44	73.15	73.30
3	73.41	73.29	73.55	73.60	73.30
7	73.92	73.38	73.56	73.65	73.90

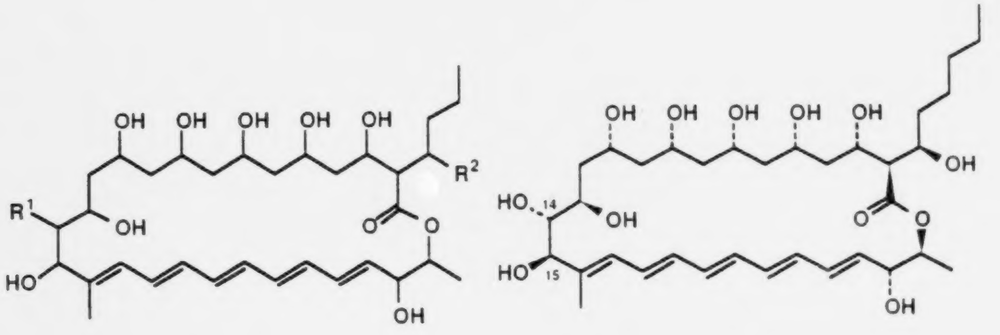
5	74.08	73.55	73.64	73.65	74.08
9	74.20	74.24	74.02	73.91	74.17
27	75.25	74.47	74.58	75.10	75.25
14	78.31	45.21	78.20	45.29	78.32
15	80.43	75.63	80.32	75.83	80.50
18	129.06	128.04	129.25	128.35	129.05
17	129.91	129.57	129.79	129.31	129.93
24	131.97	132.43	131.99	132.25	132.03
22	133.66	133.62	133.74	133.82	133.67
20	134.13	134.15	133.96	134.12	134.13
23	134.21	139.19	134.32	134.12	134.17
25	134.28	134.44	134.37	134.28	134.27
21	134.81	134.57	134.45	134.59	134.85
19	135.36	134.68	135.18	134.96	135.41
16	138.55	140.64	138.71	140.34	138.53
1	172.98	175.43	175.37	173.02	173.01

[a] 100.6 MHz ^{13}C NMR spectrum in methanol-d4 with solvent reference at 49.00 ppm.

[b] For details of spectral assignment of fungichromin (**2**) see ref. 60.

methylpentaenes (λ_{MAX} 308, 324, 342, 358 nm) indicated that these polyenes are related to chainin (**18**).[30a,47] Examination of IR and 60 MHz ^1H NMR spectra of chainin (**18**) (kindly provided by Professor K. L. Rinehart, University of Illinois) indicated that **19** possesses a very similar structure, but we could not conclusively distinguish between the two materials. Although an authentic sample of chainin (**18**) was not available, differences in optical rotation (for **19**: $[\alpha]_D^{25}$ -24.4 ° (*c* 0.16, MeOH); for **18**: $[\alpha]_D^{25}$ -112.2 ° (*c* 0.16, MeOH)) and decomposition point (for **19**: ~ 190 °C; for **18**: 222-224 °C) suggest that they may be stereoisomeric at one or more centers. We therefore designated compound **19** as isochainin (Figure 6). The other new polyenes are close relatives: 14-hydroxyisochainin (**20**), 1'-hydroxyisochainin (**21**), and 1',14-dihydroxyisochainin (**22**).

Figure 6. Structures of isochainin (**19**), 14-hydroxyisochainin (**20**), 1'-hydroxyisochainin (**21**), 1',14-dihydroxyisochainin (**22**) and pentamycin (**23**)



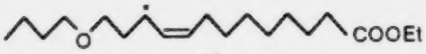
18 R1 = R2 = H
19 R1 =R2 = H
20 R1 = OH , R2 = H
21 R1 = H , R2 = OH
22 R1 = R2 = OH

23a
23b stereochemistry opposite at C-14 and C-15

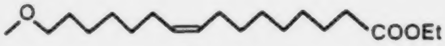
All compounds show antifungal activity roughly comparable to that of amphotericin B in preliminary tests. The great similarity in ^{13}C NMR chemical shifts and the coproduction of compounds **19**, **20**, **21**, **22** and fungichromin (**2**) suggest that the stereochemistry at every site is analogous. Recently, the absolute stereochemistry of pentamycin (**23**) (Figure 6), an antibiotic from *Streptomyces pentaticus* with the same gross structure as fungichromin (**2**), has been reported as being either **23a** or **23b**.[50] Elucidation of the stereochemical relationship between pentamycin (**23**) and fungichromin (**1**) should allow stereochemical assignment of isochainin (**19**) and its hydroxylated derivatives **20**, **21**, and **22** with reasonable confidence.

The biochemical mechanism of action of **6** is presently unknown, but it may undergo partial β-oxidation to a truncated form which interferes with either octanoate production or its attachment to the growing polyketide chain. It is interesting that no polyenes bearing phenyl groups in the side chain could be detected.

The next goal was to try to incorporate the oxa-analogues of oleate. Two oxa-oleates, ethyl (*Z*)-13-butoxytridec-9-enoate (**7**), and ethyl (*Z*)-16-methoxyhexadec-9-enoate (**8**) were synthesized by Dr. B. J. Rawlings (University of Alberta).



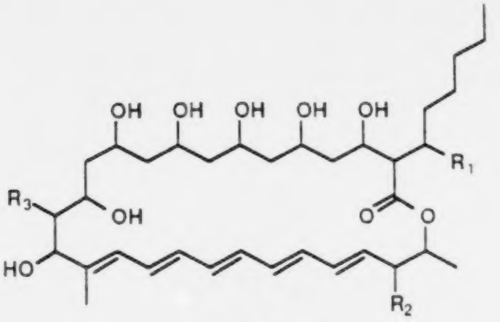
7a $\bullet = {}^{12}C$
7b $\bullet = {}^{13}C / {}^{14}C$



8

In order to determine the effects of **7a** on *S. cellulosae*, fermentations were done with different concentrations of this material, both with and without added ethyl oleate (**5**). Despite poor growth of *S. cellulosae* (small amounts of mycelia), TLC monitoring of the organic extracts still showed production of polyenes.

In subsequent studies, media with different fatty acids were employed: 1) Span 85; 2) ethyl oleate (**5**); 3) no added fatty acid; 4) 14-oxaoleate (**7a**); and medium with ${}^{13}C/{}^{14}C$ labeled 14-oxaoleate (**7b**). Because **7a** has a toxic effect on *S. cellulosae*, the amounts used for the fermentation were reduced to 66 mg/100 mL culture and the amount of ethyl oleate (**5**) was also adjusted to the same level (66 mg/100 mL). During the fermentation process, aliquots (2 mL) were removed from each of the flasks at 36 h, 60 h, 84 h, 108 h, 132 h, 156 h, and 204 h except the one containing ${}^{13}C/{}^{14}C$-labeled **7b**. The extracts from these aliquots were dissolved in MeOH (1.00 mL) and 5 μL portions of the resulting solutions were analyzed by HPLC to check for the production of polyenes. The polyenes were detected from their absorbance at 357 nm. These results are shown in Figure 7 and Figure 8.


OH
OH
OH
OH
OH
R1
R3
OH
O
O
HO
R2

fungichromin 2 $R_1 = R_2 = R_3 = OH$
filipin III 4a $R_1 = R_2 = OH$, $R_3 = H$
filipin II 4b $R_1 = R_2 = H$, $R_3 = OH$
filipin I 4c $R_1 = R_2 = R_3 = H$

Figure 7. The production of polyenes with Span 85.

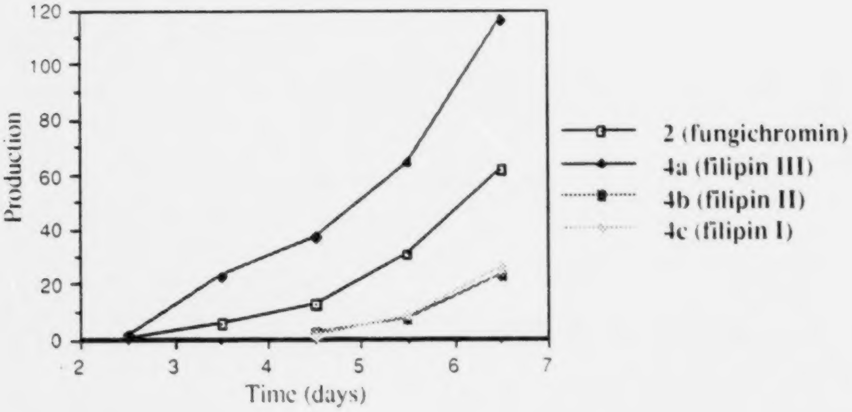
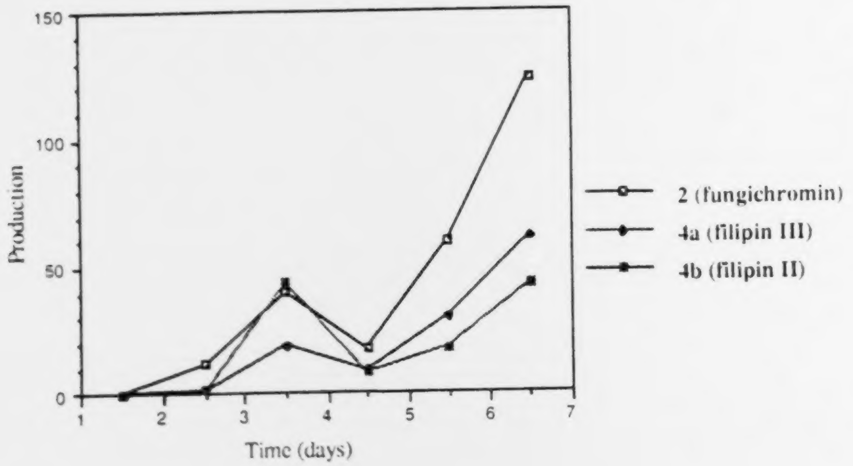


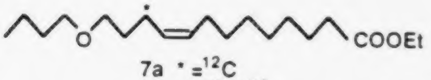
Figure 8. The production of polyenes with ethyl oleate (**5**).



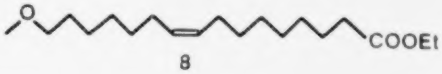
[a] The productions shown in the graphs are relative amounts and are obtained by using the amount of **2** (with Span 85) at 60 h as 1 unit; [b] All the polyenes, except for **4c**, are identified by injection of authentic samples on HPLC; [c] HPLC conditions: RP-18, $MeOH/H_2O$ = 60/40; flow rate = 0.5 mL/min; UV detection at 357 nm; retention time (R_t), 28.5 min for **2**, 36.1 min for **4a**, 45.2 min for **4b**, 50.9 min for **4c**.

For the culture with Span 85 (Figure 7), fungichromin (**2**) and filipins (**4**) were not produced in the initial lag phase (first two days). Small amounts of **2** and filipin III (**4a**) were detected in day 3. As soon as the bacterial cultures entered log phase (rapid growth), the polyene antibiotic amounts increased rapidly. On day 5, filipin II (**4b**) and filipin I (**4c**) began to accumulate. Similar results were observed for the culture with ethyl oleate (**5**). Total amounts of polyenes produced (e.g. at 6.5 days) were about the same for both Span 85 and ethyl oleate (**5**) media.

For the cultures with 14-oxaoleate (**7a**), production of **2** and **4** were greatly depressed and new polyenes were isolated. Fermentation with **8** showed that this compound was toxic to *S.cellulosae*, but less so than **7** based on the appearance of the cultures. Productions of polyenes were also low and no new polyenes could be detected by HPLC. These results indicate that both oxaoleate compounds (**7** and **8**) are toxic to



7a $^* = {}^{12}C$
7b $^* = {}^{13}C / {}^{14}C$

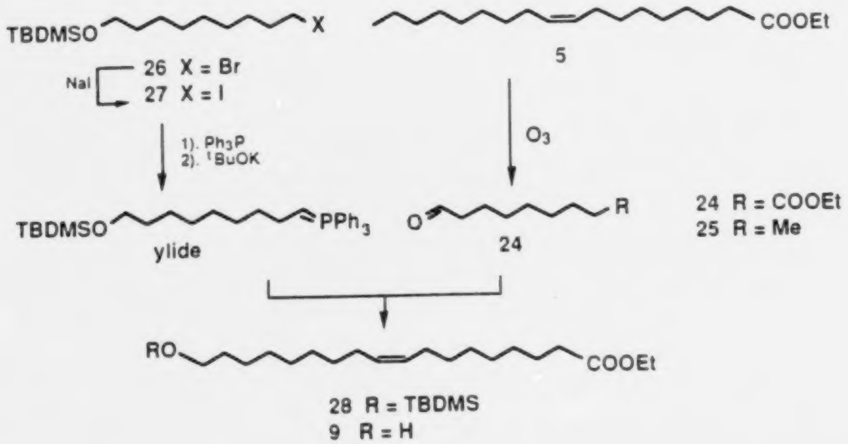


8

S. cellulosae. The biological mechanism for the observed toxicity is not known, though it may be that such oxaoleates are incorporated into lipids thereby interfering with their normal functions. This idea is supported by literature precedent: both oxa-[89] and thia-[90] fatty acids are known to be utilized in triacylglycerols and phospholipids in place of ordinary fatty acids. For example, 11-oxamyristate (10-(propoxy)decanoic) acid incorporates into the glycolipid A of *Trypanosoma brucei*, the protozoan parasite responsible for the African sleeping sickness, even more efficiently than myristate, the normal fatty acid precursor.[89] The incorporation inhibits the trypanosome growth and kills the parasite. Similarly, other oxygen analogues, 12-methoxydodecanoic acid and 5-octoxypentanoic acid, are also able to incorporate into glycolipids at least as efficiently as myristate. In contrast, palmitate, stearate, and an oxygen-substituted analogue of palmitate, 12-propoxydodecanoic acid, are not utilized. These results suggest that the specificity of fatty acid incorporation depends more on the chain length than on hydrophobicity. The production of polyene antibiotics is lipid dependent, probably because the lipid metabolites (e.g., acetates) act as precursors for antibiotic synthesis. Palmitic or oleic acid also alters the cellular fatty acid profile and changes membrane structure which may affect the influx and efflux of various amino acids.[91]

The last oleate analogue initially planned for this study was 18-hydroxyoleate (**9**). The synthesis of **9** is illustrated in Scheme 12. Ozonolysis[86] of ethyl oleate (**5**) affords ethyl 9-oxononanoate (**24**), which is required for the final Wittig reaction. The oxidation was done at -60 °C (dry-ice/CHCl$_3$) in ethanol or methanol to give **24** (65%) along with **25** as the other product in 62% yield.

Scheme 12.

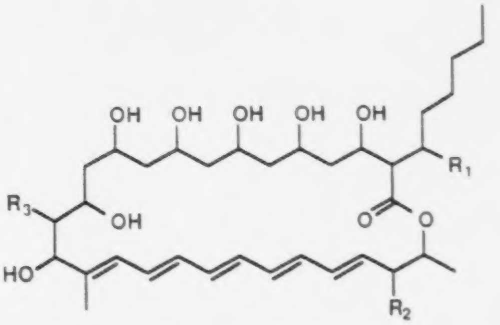

TBDMSO
X
26 X = Br
27 X = I
NaI
COOEt
5
1). Ph$_3$P
2). ^{t}BuOK
O$_3$
TBDMSO
PPh$_3$
ylide
O
R
24
24 R = COOEt
25 R = Me
RO
COOEt
28 R = TBDMS
9 R = H

The other half of the molecule was constructed as follows. Protection (65%) of the hydroxyl group of 9-bromononanol (**14**) as its TBDMS ether (**26**)[92,93] followed by halogen exchange with NaI[86] produces ethyl 9-(*tert*-butyldimethylsiloxy)nonanyl iodide (**27**) in 91% yield. Wittig condensation[87] of the triphenylphosphonium ylide derived from **27** with **24** gives a 10% yield of the desired *Z* isomer of ethyl 18-(*tert*-butyldimethylsiloxy)- oleate (**28**).

Deprotection to **9** and incorporation experiments were not done because of the low yield in the last steps and the disappointing results with other oleate anaolgues. Progress in other more promising approaches to polyketide biosynthetic studies also discouraged plans to synthesize **9** in labeled form.

Studies on the Biosynthetic Relationship of Fungichromin and Filipins

The filipins (**4**), as described in the introduction, belong to the class of pentaene antibiotics. They were first isolated from *Streptomyces filipinensis* in 1955.[59] The filipin structure[94] was first proposed to be **4a** (Figure 2) but was later found to be a mixture of four structurally similar compounds.[95]

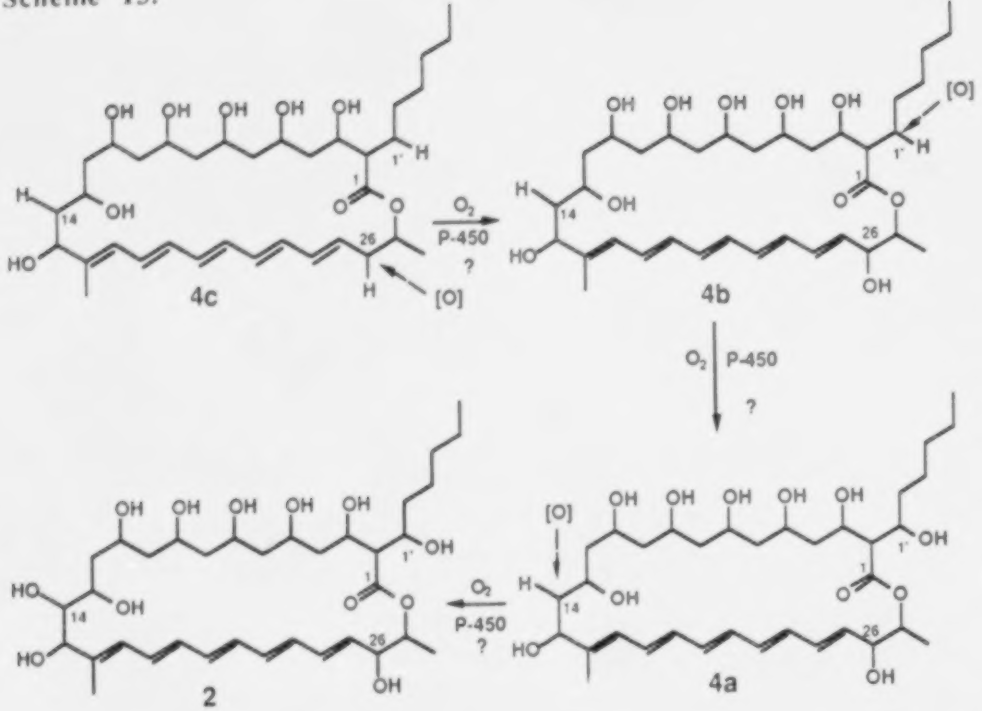


fungichromin 2 $R_1 = R_2 = R_3 = OH$
filipin III 4a $R_1 = R_2 = OH$, $R_3 = H$
filipin II 4b $R_1 = R_2 = H$, $R_3 = OH$
filipin I 4c $R_1 = R_2 = R_3 = H$

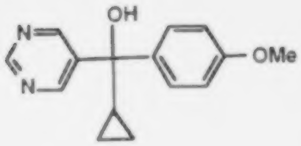
Bergy and Eble showed that crystalline filipin can be resolved into filipin I, filipin II, filpin III, and filipin IV, which constitute 4, 25, 53, and 18%, respectively, of the original material.[95] Filipin I was reported to be a heptahydroxy, filipin II an octahydroxy, and filipin III and IV nonahydroxy compounds by Pandey and Rinehart.[96] Recently, from Edward's study on filipin complex by direct liquid introduction LC-MS, the structure of filipin II (**4b**) was proposed to be the 1'-deoxy-derivative of filipin III (**4a**).[97]

Since filipins (**4**) are coproduced with fungichromin (**2**) by *S. cellulosae*, they are almost certainly biosynthetically related. For example, **2** could originate from **4a** by insertion of a hydroxyl group at C-14. Similarly **4a** could arise by oxidation of **4b** (Scheme 13). One approach to test this hypothesis is to feed a less oxidized polyene (e.g., **4a**) in labeled form to *S. cellulosae* and then isolate the more oxidized products (e.g., **2**).

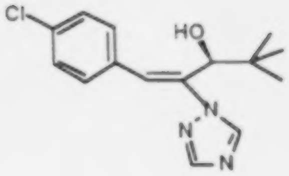
Scheme 13.



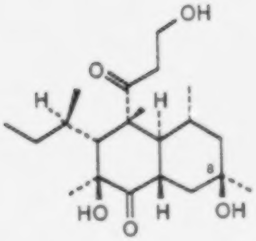
Since the production of **4b** and **4c** are normally low, a method was sought to increase the yields of these compounds. Hydroxylation is an important step during later stages of the biosyntheses of many natural products such as erythromycin,[98] rifamycin[99] and trichothecene.[100] The enzymes involved in these reactions are commonly believed to be cytochrome P-450 proteins. The P-450 enzymes have been investigated extensively,[101] and inhibitors have been examined in biosynthetic or metabolic pathways in plants[102,103] and mammals.[101,104] Studies utilizing P-450 enzyme inhibitors in polyketide biosynthesis were reported recently by Japanese researchers.[105,106] Their work shows that when ancymidol (**29**), or S-3307 ((*E*)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol) (**30**) are added to cultures of *Phoma betae* that produces betaenenone B (**31**), a deoxygenated intermediate **32**, which is not normally present in large amounts, is formed in substantial quantity.



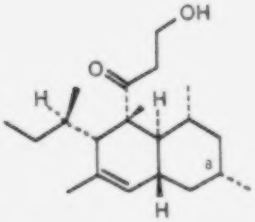
29



30



31 betaenone B



32 intermediate

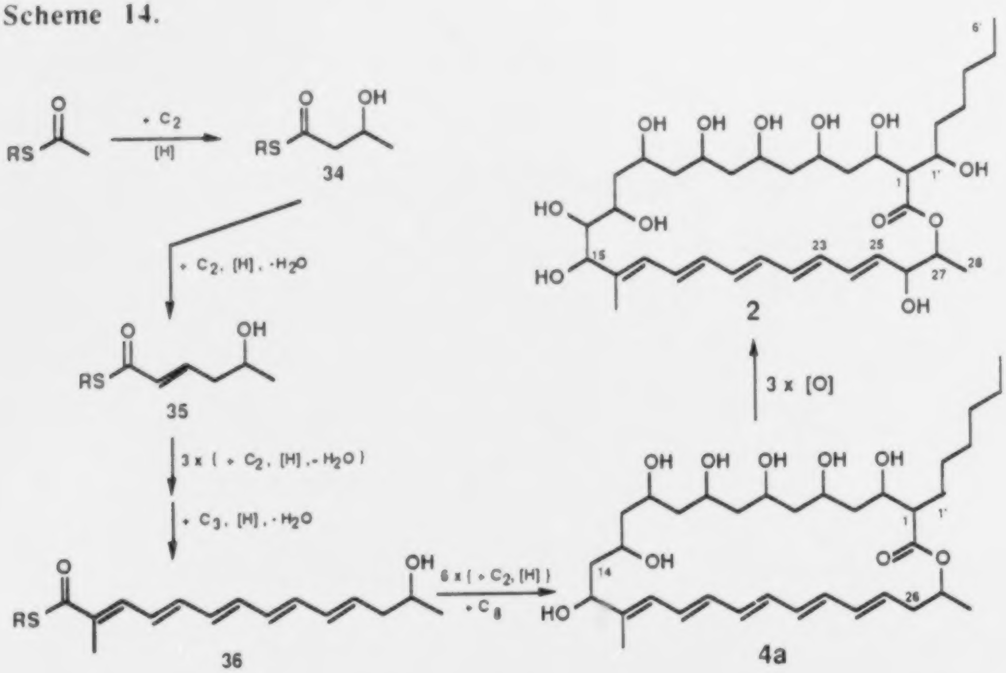
In an attempt to enhance the production of labeled filipins to be tested as precursors of fungichromin (**2**), *S. cellulosae* cultures were treated with ancymidol (**29**). Filipin III (**4a**) and filipin II (**4b**) were isolated and purified. Addition of labeled **4a** to *S. cellulosae* gave a labeled fungichromin (**2**), while a sample of **4b** from the same culture was not labeled. This result shows that fungichromin (**2**) is biosynthetically derived from the hydroxylation of filipin III (**4a**) at C-14.

Because *S. cellulosae* did not produce filipin I (**4c**), an alternative method using *S. filipinensis* was examined. Unfortunately, the cultures of *S. filipinensis* in the presence of the P-450 inhibitors (**29** and **30**) failed to give sufficient amounts of filipin I (**4c**) for us to continue the studies.

Syntheses and Incorporation of Advanced Precursors into Fungichromin

The biosynthesis of fungichromin (**2**) is believed to start with acetyl-CoA at the hydroxyl end of the lactone ring (C-27 to C-28), which upon condensation with malonyl-CoA and reduction, gives the 3-hydroxybutryate (**34**) (Scheme 14). According to the polyketide hypothesis, this process is repeated to produce the 6-carbon unit **35**, the pentaene **36**, and eventually filipin I (**4a**). This is then presumably oxidized to fungichromin (**2**). Incorporations of precursors such as **34**, **35**, and **36** (Scheme 14) would provide direct evidence to support this hypothesis.

Scheme 14.

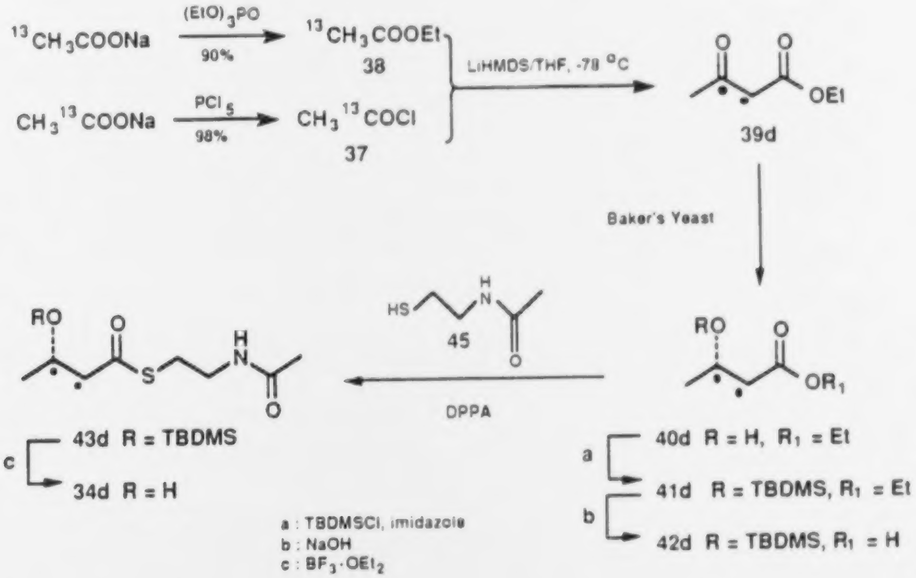

RS
O
+ C2
[H]
O
OH
RS
34
+ C2, [H], -H2O
O
OH
RS
35
3 x (+ C2, [H], - H2O)
+ C3, [H], - H2O
O
OH
RS
36
6 x (+ C2, [H])
+ C8
6'
OH
OH
OH
OH
OH
1
1'
OH
HO
OH
O
O
15
23
25
27
28
HO
OH
2
3 x [O]
OH
OH
OH
OH
OH
1
1'
OH
14
O
O
26
HO
4a

Unfortunately, catabolism of precursors larger than acetate or propionate is a common problem with whole cell studies of polyketide biosynthesis. *N*-acetylcysteamine (NAC) thioesters of propionate-derived diketides have been shown to be incorporated successfully into tylactone[13] and erythromycin,[12] where the corresponding acids or normal esters are completely degraded by β-oxidation. These results prompted us to target the synthesis of the NAC thioester derivatives of **34**, **35** and **36** as potential precursors for incorporation experiments with **2**.

Throughout the following text, the letter (**a**, **b**, **c**, **d**, **e**, or **f**) after a number refer to an unlabeled racemic (**a**), unlabeled optical active (**b**), monolabeled (**c**), doubly labeled (**d**), triply labeled (**e** or **f**) compound.

Synthesis of NAC [2,3-$^{13}C_2$]-(*S*)-3-hydroxybutyrate (**34d**) could be achieved by two methods. In the first method (Scheme 15), designed by Dr. Y. Yoshizawa of our group, labeled sodium [1-^{13}C]acetate (isotopic purity 99% ^{13}C) was converted to the corresponding acetyl chloride (**37**) in 98% yield.[107] Sodium [2-^{13}C]acetate (isotopic purity 99% ^{13}C) was treated with triethylphosphate to give ethyl [2-^{13}C]acetate (**38**) (90%).[108] The enolate resulting from the reaction of **38** with LiHMDS was treated with **37** to afford ethyl [2,3-$^{13}C_2$]acetoacetate (**39d**) (63%).[109,110] Protection[111] of the

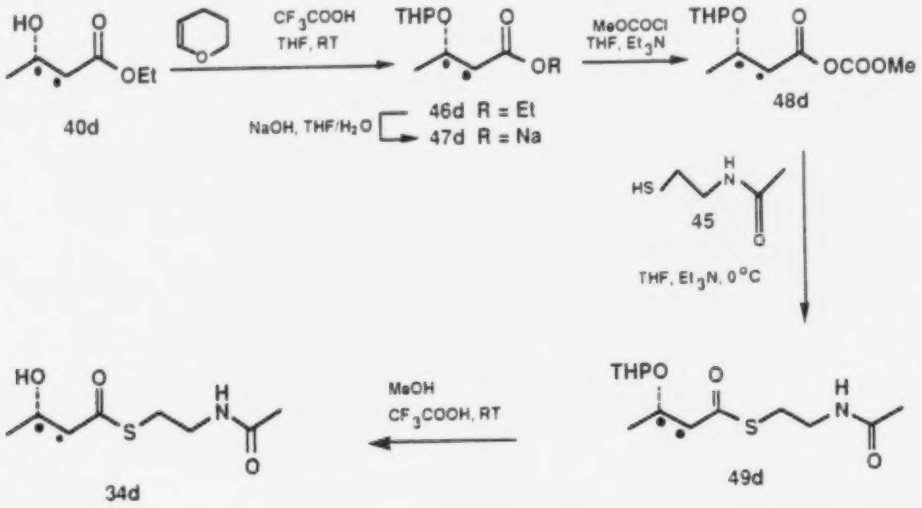
Scheme 15.


$^{13}CH_3COONa$
$(EtO)_3PO$
90%
$^{13}CH_3COOEt$
38
$CH_3{}^{13}COONa$
PCl_5
98%
$CH_3{}^{13}COCl$
37
LiHMDS/THF, -78 °C
OEt
39d
Baker's Yeast
RO
OR_1
40d R = H, R_1 = Et
41d R = TBDMS, R_1 = Et
42d R = TBDMS, R_1 = H
HS
45
DPPA
43d R = TBDMS
34d R = H
a : TBDMSCl, imidazole
b : NaOH
c : $BF_3 \cdot OEt_2$

hydroxyl group of ethyl [2,3-$^{13}C_2$]-(*S*)-3-hydroxybutyrate (**40d**), obtained in 89% yield from Baker's yeast reduction of **39d**,[112] gave silyl ester **41d**. Saponification (80%) to **42d** followed by esterification (72%) with *N*-acetylcysteamine (**45d**)[113] and DPPA gave the protected NAC thioester **43d**.[114] Finally, removal[115] of the silyl protecting group with boron trifluoride etherate yielded the desired labeled diketide precursor **34d** (44%) (isotopic purity 99% $^{13}C_2$; optical purity 90% ee. Determination of the optical purity of **34d** will be discussed after the synthesis of the tetraketide **82d**).

In the second approach (Scheme 16),[110] instead of a silyl protecting group, the tetrahydropyranyl (THP) group was used to protect the hydroxy group of **40d**. Treatment of compound **40d** with 3,4-dihydro-2*H*-pyran in the presence of a catalytic amount of trifluoroacetic acid gave the THP ether **46d** in quantitative yield. Hydrolysis of the ester group of **46d** with sodium hydroxide afforded the sodium salt **47d**, which was converted directly to the mixed anhydride **48d** by reaction with methyl chloroformate in the presence of triethylamine. Reaction of the mixed anhydride **48d** with *N*-acetylcysteamine (**45**)

Scheme 16.

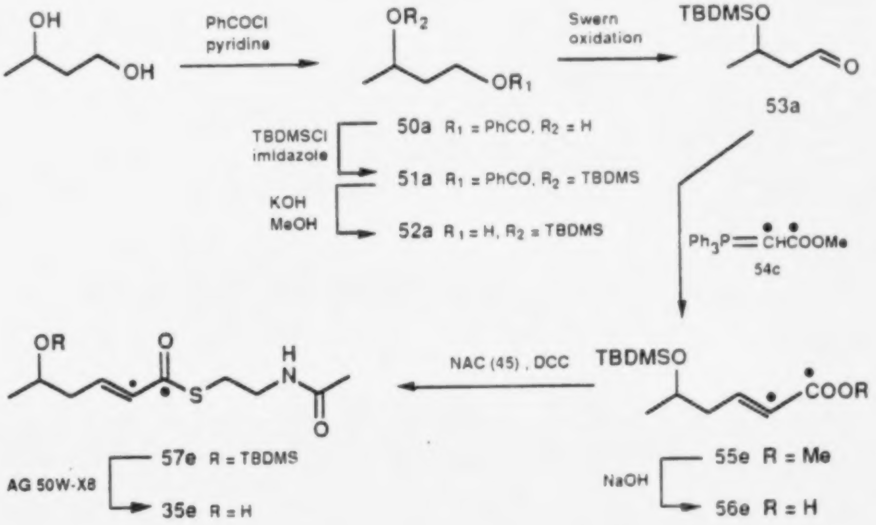

HO
O
OEt
40d
CF3COOH
THF, RT
THPO
O
OR
46d R = Et
47d R = Na
NaOH, THF/H2O
MeOCOCl
THF, Et3N
THPO
O
OCOOMe
48d
HS
H
N
O
45
THF, Et3N, 0°C
THPO
O
S
H
N
O
49d
MeOH
CF3COOH, RT
HO
O
S
H
N
O
34d

followed by removal of the THP group with trifluoroacetic acid in methanol gave **34d** (32% overall yield from **40d**) (isotopic purity 99% $^{13}C_2$; optical purity 90% ee).

The doubly ^{13}C-labeled compound **34d** can be obtained conveniently by both methods. In the first approach (Scheme 15), all reactions generally give good yields, except for the deprotection of the silyl ether by $BF_3{\cdot}OEt_2$ (**44d** to **34d**), which can be problematic. In the second method, all the reactions proceed quite well provided care is taken with the acid labile THP protecting group.[116]

The second target compound, NAC 5-hydroxyhex-2-enoate (**35e**) was synthesized as illustrated in Scheme 17. Selective reaction[111] of the primary hydroxyl group of racemic 1,3-butanediol with benzoyl chloride gave the benzoyl ester **50a** (82%).

Scheme 17.

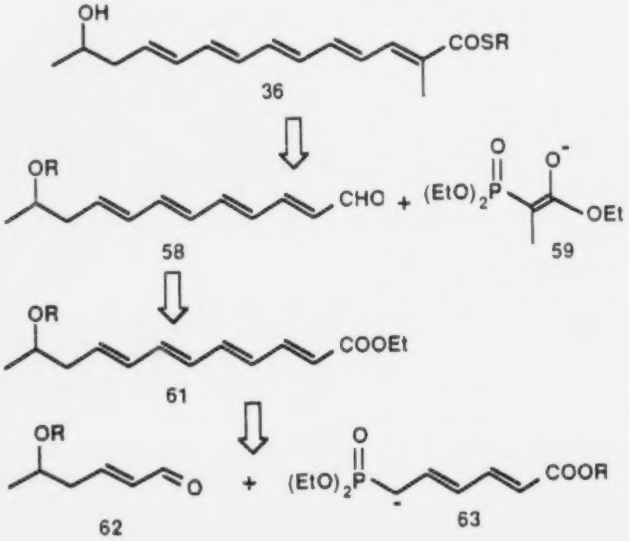


OH
OH
PhCOCl
pyridine
OR2
OR1
Swern
oxidation
TBDMSO
O
53a
TBDMSCl
imidazole
50a R1 = PhCO, R2 = H
51a R1 = PhCO, R2 = TBDMS
KOH
MeOH
52a R1 = H, R2 = TBDMS
Ph3P=CHCOOMe
54c
OR
O
H
N
S
O
NAC (45), DCC
TBDMSO
COOR
57e R = TBDMS
AG 50W-X8
35e R = H
55e R = Me
NaOH
56e R = H

The tertiary hydroxyl group was protected by *tert*-butyldimethylsilyl (TBDMS) chloride to afford **51a** in quantitative yield. Hydrolysis[111] of the benzoate with potassium hydroxide yielded the alcohol **52a**, which was then converted to the aldehyde **53a** by Swern oxidation (89%).[117,118] Wittig reaction of **53** with **54c**[119] gave the α,β-unsaturated methyl ester **55e** (70%). Hydrolysis of the methyl ester with sodium hydroxide[111] (71%) followed by esterification of the resulting acid **56e** using DCC[120] gave NAC thioester **57e** (39%). Removal[121] of the silyl group with AG 50W-X8 (Bio Rad) cation exchange (H^+) resin in methanol produced the desired racemic triketide **35e** in 74% yield.

The synthesis of the 15-carbon precursor **36**, 13-hydroxy-2-methyl-2,4,6,8,10-tetradecapentaene, could be accomplished by at least two methods. In the first method (Scheme 18), the known allylic aldehyde **62**[111] could react with the known Horner-Emmons reagent **63**[41b] to yield a conjugated tetraene ester **61**, which could then be reduced and reoxidized to the corresponding tetraene aldehyde **58**. Coupling of **58** with the known Horner-Emmons reagent **59**[119] should give the desired pentaene **36**.

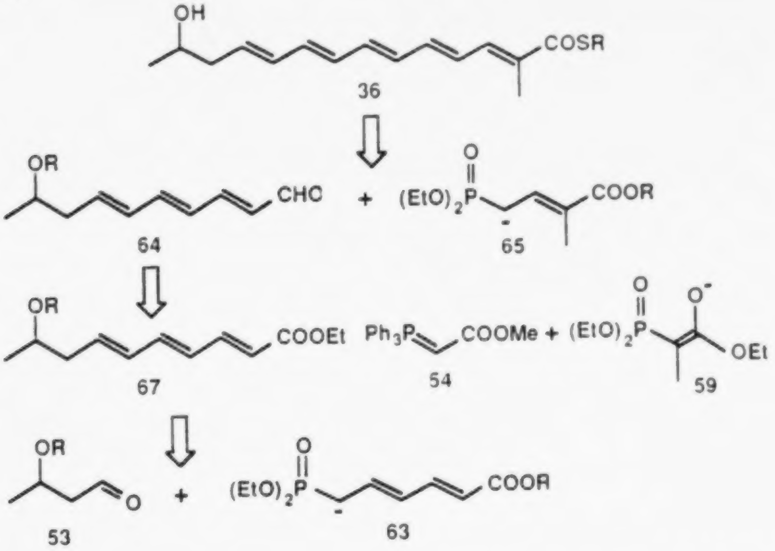
Scheme 18.



In the second approach (Scheme 19), the pentaene **36** would be constructed by the reaction between the triene aldehyde **64** and the anion **65**, which is derived from 4-bromo-2-methylcrotonaldehyde.[123] Alternatively, the aldehyde **64** could first react with ylide **54**, and then **59** could be added after functional group modification to produce the final product

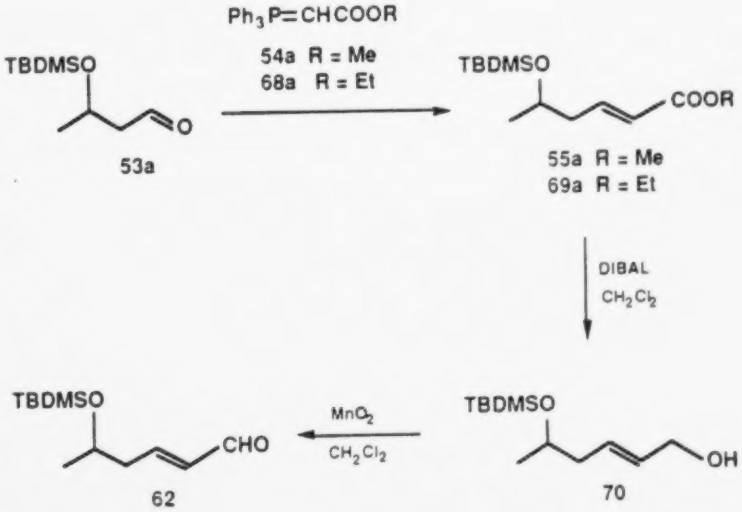
36. In this method, two ^{13}C labels could easily be placed into the C-2 and C-3 positions of **36** by using [1-^{13}C]-labeled **54** and [2-^{13}C]-labeled **59**.

Scheme 19.


OH
COSR
36
OR
CHO
+
(EtO)$_2$P
O
COOR
64
65
OR
COOEt
Ph$_3$P
COOMe
+
(EtO)$_2$P
O
O$^-$
OEt
67
54
59
OR
O
+
(EtO)$_2$P
O
COOR
53
63

The allylic aldehyde **62** (R = TBDMS) was generated by using Seebach's method (Scheme 20).[111] The 5-(*tert*-butyldimethylsiloxy)hexenoate (**55a** or **69a**) was obtained by

Scheme 20.

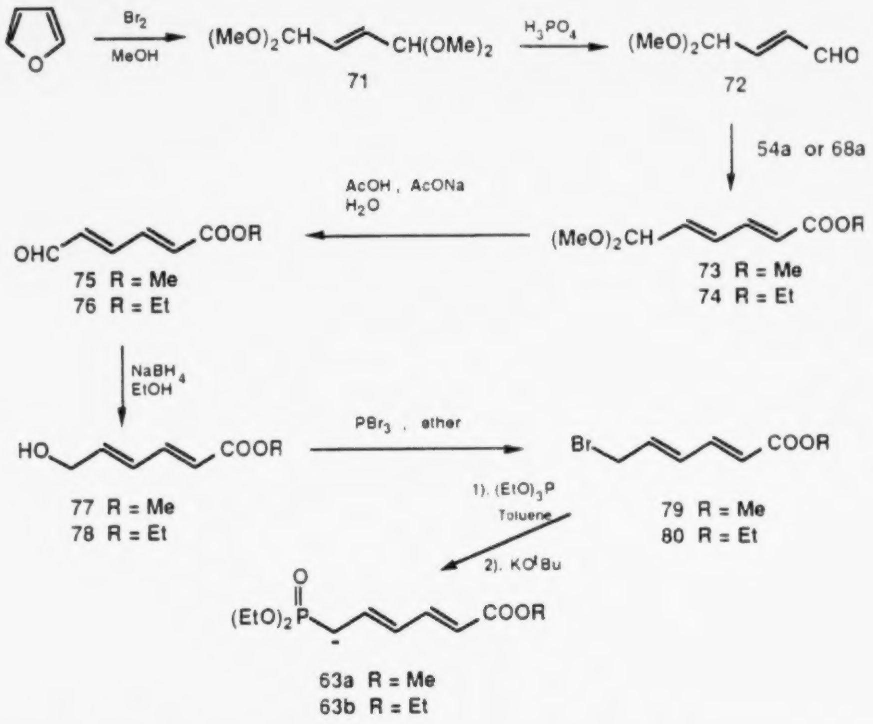

Ph$_3$P=CHCOOR
54a R = Me
68a R = Et
TBDMSO
O
53a
TBDMSO
COOR
55a R = Me
69a R = Et
DIBAL
CH$_2$Cl$_2$
TBDMSO
CHO
62
MnO$_2$
CH$_2$Cl$_2$
TBDMSO
OH
70

using the same procedures described in Scheme 17. The ester group was then reduced by DIBAL to generate the allylic alcohol (**70**) (93%), which was oxidized by manganese dioxide (MnO_2) to the desired aldehyde **62** in 83% yield. Commercially available MnO_2 was inactive in the oxidation reaction; however, active MnO_2 was prepared by a literature procedure[124] and could be stored for many months in a refrigerator without losing its activity.

The Horner-Emmons reagent **63** is derived from 6-bromosorbate. It is a versatile reagent for the synthesis of conjugated double bond systems and has been used in the total synthesis of amphotericin B.[12b] It appeared likely that direct bromination of the allylic methyl (6-methyl) group of commercially available ethyl sorbate would readily produce 6-bromosorbate. However, this reaction was found to be more complex than we had imagined: bromination using the literature procedure[125,126] gave a mixture of products resulting from attack at the 6-methyl group and at the double bonds. Consequently, De Koning's method was followed (Scheme 21).[122a]

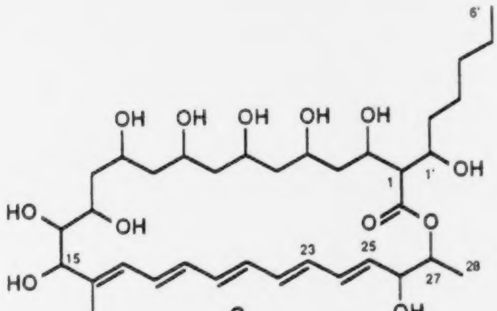
Furan reacts with bromine in methanol at -45 °C to give the bisacetal 1,1,4,4-tetramethoxy-2-butene **71** (78%).[122b] The removal of two methoxy groups at the one end of the bisacetal **71** under acidic conditions produces 4,4-dimethoxycrotonaldehyde (**72**) (72%).[122c] Wittig reaction of aldehyde **72** with **54a** affords the conjugated diene ester **73** (64%).[122a] Since compounds **71**, **72** and **73** contain dimethoxy acetal groups, they are acid labile. Trace amounts (2-10%) of the deprotected aldehydes were detected in the ^1H NMR spectra. Deprotection of the acetal group of **73** with aqueous acetic acid affords the conjugated aldehyde **75** as a yellow solid (61%). Reduction of the aldehyde group of **75** by $NaBH_4$ in ethanol gives the desired methyl 6-hydroxysorbate (**77**) along with the undesired ethyl 6-hydroxysorbate (**78**) as a 2:1 mixture (quantitative yield). The latter compound results from the attack of ethoxy anion generated from the basic solution on the methyl ester group of sorbate. Treatment of the mixed alcohol (**77** and **78**) with phosphorus tribromide yields the 6-bromosorbates (**79** and **80**) (79%). The

Scheme 21.

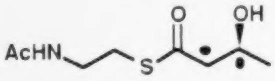

Br2
MeOH
(MeO)2CH CH(OMe)2
71
H3PO4
(MeO)2CH CHO
72
54a or 68a
(MeO)2CH COOR
73 R = Me
74 R = Et
AcOH, AcONa
H2O
OHC COOR
75 R = Me
76 R = Et
NaBH4
EtOH
HO COOR
77 R = Me
78 R = Et
PBr3, ether
Br COOR
79 R = Me
80 R = Et
1). (EtO)3P
Toluene
2). KOtBu
(EtO)2P COOR
63a R = Me
63b R = Et

6-bromosorbates react with triethylphosphate, and treatment of the resulting phosphonate with potassium *tert*-butoxide generates the anion **63** *in situ*.[41b] This could be then coupled with the aldehyde **62**.

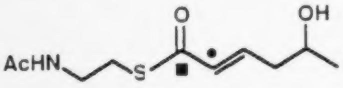
Since the Horner-Emmons reagents **63a** and **63b**, having mixed methyl/ethyl ester groups were used in the last reaction for making **61**, the products were difficult to separate. However, the ^1H NMR spectra of the products indicated the formation of the required conjugated double bonds. In order to avoid the formation of mixed esters in future studies, the reduction of **75** should be carried out in methanol. Alternatively, **76** could be used. This compound should be easily obtained from the condensation between **72** and **68a**. At this point in the preparation of the advanced precursors to fungichromin (**2**), feeding experiments with the precursors **34d** and **35e** were performed under various conditions. Samples of labeled fungichromin (**2**), isolated after the administration of **34d** or **35e** to cultures of *S. cellulosae*, gave only non-specific enhancements of their ^{13}C NMR signals. Thus, no intact incorporation of these precursors had been achieved.



2



34d



35e

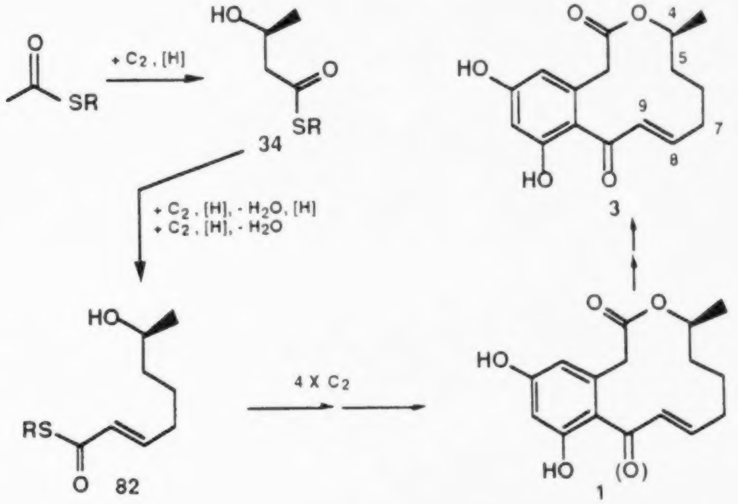
The difficulties with intact incorporation of the di- and triketides into polyketides cast doubt on the feasibility of successful utilization of large precursors such as **36**. Therefore, rather than completing the synthesis of this labeled precursor, attention was focused on the problem of preventing degradation of advanced intermediates by whole cell systems.

BIOSYNTHETIC STUDIES ON DEHYDROCURVULARIN AND INCORPORATIONS OF ADVANCED PRECURSORS

Introduction: Intact Incorporation of Advanced Polyketide Intermediates

As described in the introduction, the biosyntheses of polyketides resemble those of fatty acids, and it is believed that the polyketide is assembled in a stepwise manner. The oxidation level and the stereochemistry of the growing polyketide chain are adjusted after each condensation step, and prior to the addition of the next extender unit (malonyl-, methylmalonyl-, or ethylmalonyl-CoA).[127] According to this hypothesis, dehydrocurvularin (**3**) is formed as shown in Scheme 22.[74]

Scheme 22.



One approach to test this hypothesis is to incorporate the putative intermediates (e.g., **34** or **82**) which have an identical stereostructure and oxidation state to the polyketide metabolite (**3**). Low molecular weight acids such as acetic acid, propionic acid, and butyric acid are easily incorporated into polyketide metabolites. Except for a few cases where the precursors serve as the starter units,[128] attempts to incorporate precursors larger than 4-carbon atoms often fail due to the rapid catabolism of the precursors by β-oxidation.[129] As a result, only incorporation of the label as acetate/malonate is observed experimentally.[129] Several intact incorporations of propionate derived di- and triketides have been reported recently;[12-15] however, in all these experiments, major portions of the labeled precursors were degraded by β-oxidation back to propionate prior to their incorporation into the metabolites. As seen in the previous section, attempts to incorporate *acetate-derived* precursors such as **34d** or **35e** into fungichromin (**2**) failed because of degradation back to acetates. Thus, the enzymes of β-oxidation would have to be suppressed in order to achieve the desired intact incorporation of advanced precursors.

The β-Oxidation Pathway

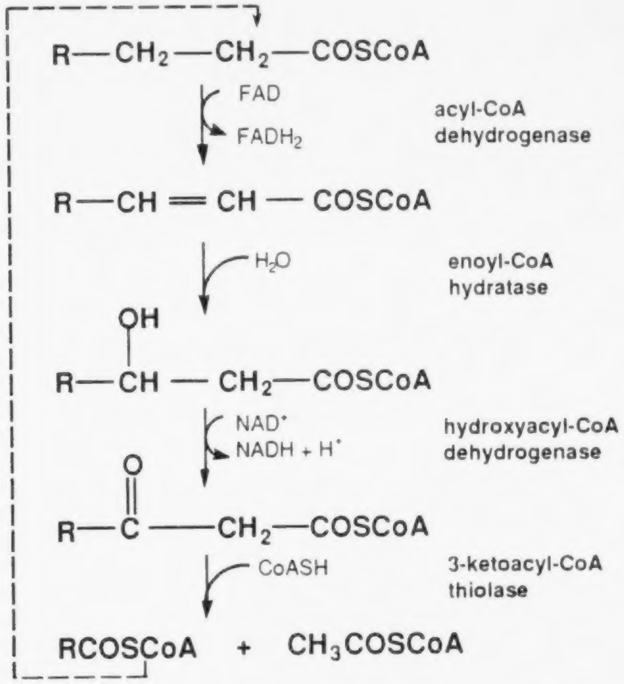
β-Oxidation is a common pathway for the degradation of saturated and unsaturated fatty acids in eukaryotes and prokaryotes.[130] Fatty acids, stored as triacylglycerols, are the main fuel reserves of animals and are readily used by most tissues, with a few specialized exceptions such as nerve cells and erythrocytes. This degradation involves the successive oxidative removal of acetyl groups from the carboxyl end of long chain fatty acids.[130,131] It was believed to occur only in the mitochondrial matrix in animal tissues until Lazarow and de Duve reported the presence of a β-oxidation system in rat liver peroxisomes in 1976.[132] Research interest in this area has been growing constantly since the identification of this system and the discovery of several inherited human diseases due to deficiencies in β-oxidation enzymes.[133-138]

In the first step of the process, fatty acid esters can be hydrolyzed to the free fatty acids by the catalytic effect of lipases. The free fatty acids are then transported across the plasma membrane through the mediation of the membrane fatty acid binding proteins,[139-142] or by a spontaneous and nonspecific diffusion across the plasma membrane.[143,144]

The first of four reactions in the β-oxidation series is the dehydrogenation of acyl-CoA to 2-*trans*-enoyl-CoA catalyzed by acyl-CoA dehydrogenase (Scheme 23). These enzymes can be classified into three types according to their chain length specificities: short chain, medium chain, and long chain dehydrogenases.[145-148] The cooperation of short chain dehydrogenase, which acts on butyryl-CoA and hexanoyl-CoA, with medium chain dehydrogenase, which is highly active toward substrates from hexanoyl-CoA to dodecanoyl-CoA, and the long chain dehydrogenase, which preferentially acts on octanoyl-

CoA and longer chain substrates, assures high rates of dehydrogenation over the whole spectrum of normal fatty acids and their chain shortened intermediates.

Scheme 23.


R—CH2—CH2—COSCoA
FAD
FADH2
acyl-CoA dehydrogenase
R—CH=CH—COSCoA
H2O
enoyl-CoA hydratase
OH
R—CH—CH2—COSCoA
NAD+
NADH + H+
hydroxyacyl-CoA dehydrogenase
O
R—C—CH2—COSCoA
CoASH
3-ketoacyl-CoA thiolase
RCOSCoA + CH3COSCoA

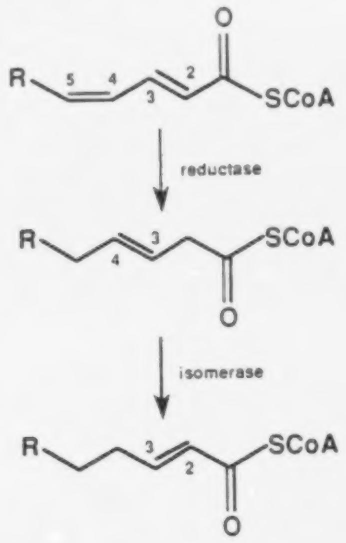
The dehydrogenases are the most studied proteins among all the β-oxidation enzymes. Structurally, acyl-CoA dehydrogenases, with molecular weights between 170,000 and 190,000, are homotetramers containing one equivalent of flavin adenine dinucleotide (FAD) per subunit.[149-153] The dehydrogenation of acyl-CoA thioesters occurs with loss of one α- and one β-hydrogen to form the α,β-enoyl-CoA thioesters with concomitant reduction of the FAD to $FADH_2$. The re-oxidation of the $FADH_2$ to FAD is a very complex process that involves several other mitochondrial flavoproteins and electron transfers.[154,155]

The mechanism for the reductive half reactions in the dehydrogenation has been extensively studied but is still not fully understood.[156-167] The generally accepted catalytic mechanism of the dehydrogenases involves initial abstraction of the *pro*-R-proton at the α-carbon of the substrate and hydride transfer of the *pro*-R-hydrogen at the β-carbon of the substrate to the N-5 position of the flavin.

The second reaction of the β-oxidation process is the hydration of 2-*trans*-enoyl-CoA to L-3-hydroxyacyl-CoA catalyzed by the soluble matrix enzyme enoyl-CoA hydratase.[131,168] The third step is the dehydrogenation of L-3-hydroxyacyl-CoA by L-3-hydroxyacyl-CoA dehydrogenase to 3-ketoacyl-CoA.[131,168] The final step is the thiolytic cleavage of the 3-ketoacyl-CoA thioester to an acyl-CoA chain shortened by two carbon atoms and acetyl-CoA.[131,168]

For unsaturated fatty acids, two additional enzymes are required to complete their degradation by β-oxidation (Scheme 24). The enzymes are: 2,4-dienoyl-CoA reductase and Δ^3-*cis*-Δ^2-*trans*-enoyl-CoA isomerase.[169] The first enzyme catalyses the NADPH-dependent 1,4-addition of hydrogen across the 2,4-diene system; the remaining double bond appearing in the 3-position. The 3 double bond resulting from this reductive

Scheme 24.

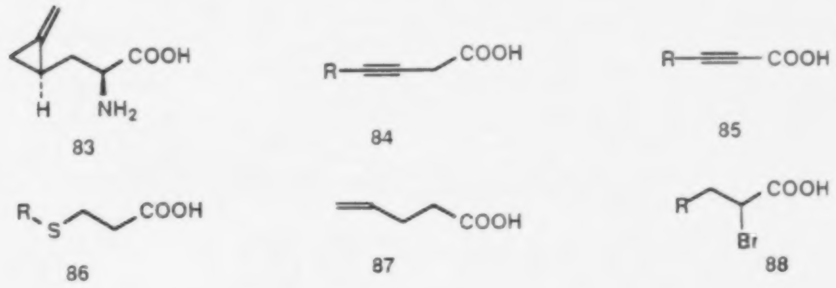


stage of β-oxidation is subsequently isomerized to the *trans*-2-position by Δ^3-*cis*-Δ^2-*trans*-enoyl-CoA isomerase,[169,170] thus allowing β-oxidation to proceed in the conventional manner.

Introduction to Inhibition of β-Oxidation

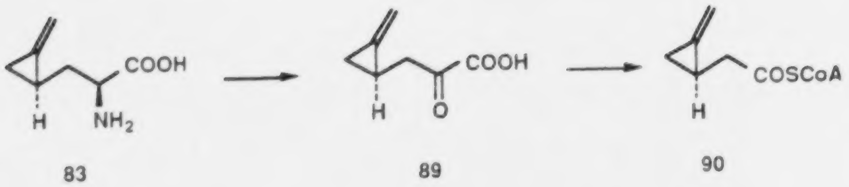
Hypoglycin A (**83**)[171] (Figure 9) is probably the first example of a β-oxidation inhibitor, and its mechanism of action has been studied for more than 35 years. This unusual amino acid, (+)-2-amino-2-methylenecyclopropanepropionic acid (**83**), is present in unripe ackee fruit and is the causative agent for Jamaican Vomiting Sickness.[172-174] While the ripe fruit serves as a dietary staple in Jamaica, ingestion of the unripe fruit causes severe hypoglycemia and often death in man. During the efforts to examine mammalian β-oxidation systems, many other inhibitors were discovered. Some of these compounds are shown in Figure 9.

Figure 9. Structures of some β-oxidation inhibitors



Hypoglycin A (**83**) is transaminated in animal cells to methylenecyclopropane pyruvic acid (**89**) (Scheme 25) which is then oxidatively decarboxylated to (*R*)-2-methylenecyclopropane acetyl-CoA (MCPA-CoA) (**90**), an irreversible inhibitor of several

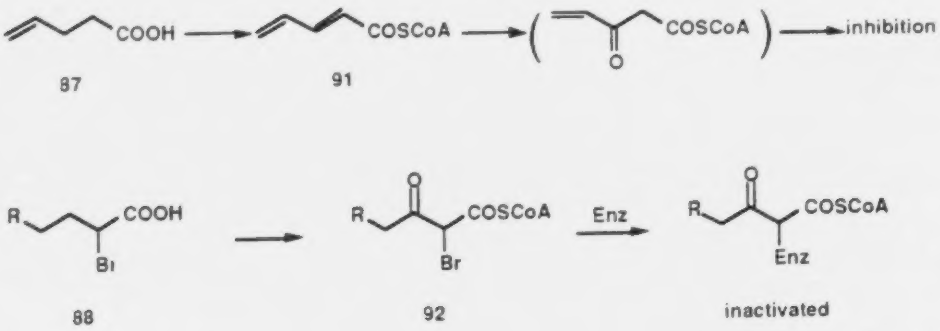
Scheme 25.


COOH
H
NH$_2$
83
COOH
H
O
89
COSCoA
H
90

acyl-CoA dehydrogenases.[175,176] The inhibition of acyl-CoA dehydrogenase by MCPA-CoA is believed to proceed via a radical process that involves the opening of the methylenecyclopropyl ring of MCPA-CoA (**90**) and formation of a covalent adduct with the flavine adenine dinucleotide, thus inactivating the enzyme.[177,178]

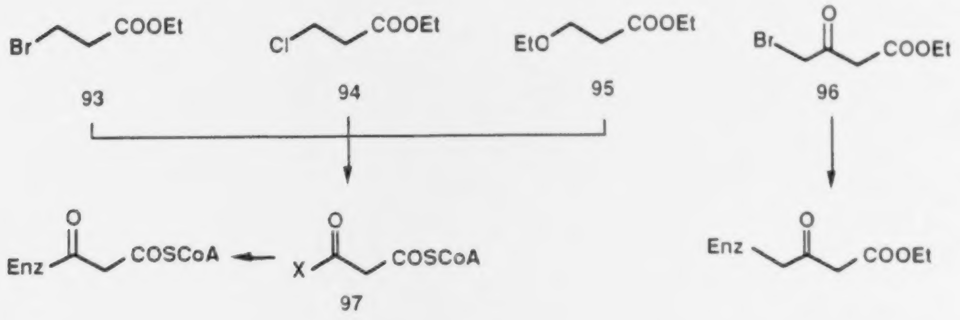
4-Pentenoic acid (**87**, Scheme 26), the first recognized 3-ketoacyl-CoA thiolase inhibitor, was discovered during structure-activity studies on hypoglycin (**83**). It is activated to 4-pentenoyl-CoA in animal cells, and then dehydrogenated to 2,4-pentadienoyl-CoA (**91**) (Scheme 26),[176] an inhibitor of 3-ketoacyl-CoA thiolase. Similarly, 2-bromooctanoic acid (**88**, R = C_4H_9, Scheme 26) is metabolized to **92**; it is believed that subsequent nucleophilic displacement of bromide results in the inactivation of the thiolase.[176]

Scheme 26.


COOH
87
COSCoA
91
O
COSCoA
inhibition
R
COOH
Br
88
R
O
COSCoA
Br
92
Enz
R
O
COSCoA
Enz
inactivated

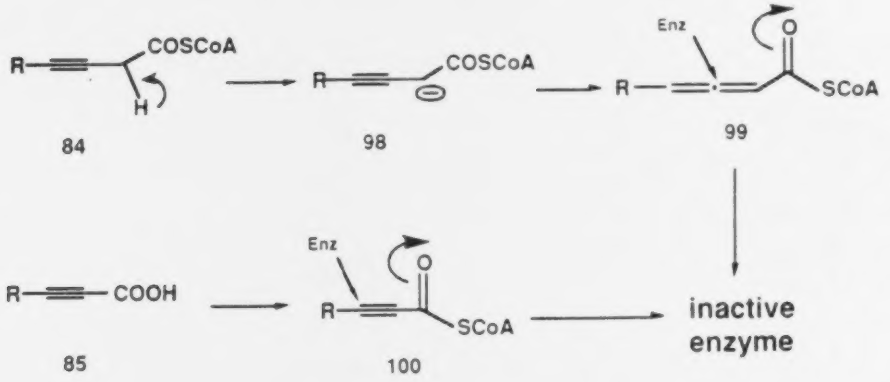
Some other potential β-oxidation inhibitors (**93**, **94**, **95**, and **96**) were designed and synthesized in our laboratory; the rationale for possible enzyme inhibition by these compounds is presented in Scheme 27.

Scheme 27.


Br
COOEt
93
Cl
COOEt
94
EtO
COOEt
95
O
Br
COOEt
96
O
Enz
COSCoA
O
X
COSCoA
97
O
Enz
COOEt

The 2- and 3-alkynoic acid derivatives (**84** and **85**, Scheme 28) are also inhibitors of acyl-CoA dehydrogenase.[179] Deprotonation at the α-position of **84** by the dehydrogenase would result in the anion **98**, which could isomerize to the reactive allenic compound **99**. The double bond in the allenic intermediate is conjugated with the carbonyl group, and thus **99** behaves as a strong Michael acceptor. Nucleophilic attack on the conjugated allene by the enzyme would result in enzyme inactivation (Scheme 28).[159,180]

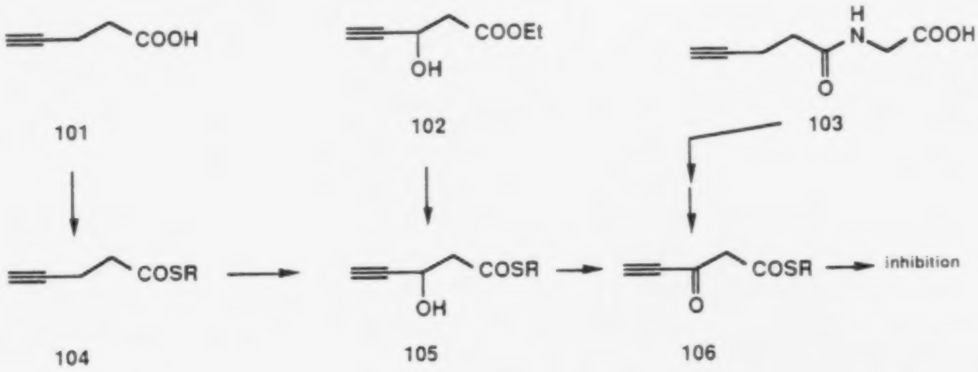
Scheme 28.


R
COSCoA
H
84
R
COSCoA
98
Enz
O
R
SCoA
99
R
COOH
85
Enz
O
R
SCoA
100
inactive enzyme

Esterification of the 2-alkynoic acid **85** to an akynoyl-CoA derivative would activate the triple bond, and the Michael attack by the enzyme on the triple bond would inhibit the enzyme (Scheme 28).[181]

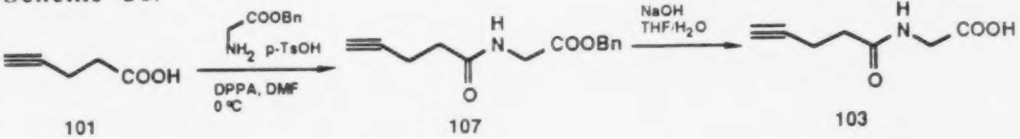
From the inhibition studies that will be discussed below, we found that the alkynoic acid type compounds generally give better inhibition results. 4-Pentynoic acid (**101**), ethyl 3-hydroxy-4-pentynoate (**102**) and the 4-pentynoic acid derivative (**103**) (Scheme 29) were also used in our studies. This class of compounds may be metabolized to the activated form **106**, which could react with and disable the enzyme.

Scheme 29.


COOH
101
COOEt
OH
102
H
N
COOH
O
103
COSR
104
COSR
OH
105
COSR
O
106
inhibition

The 4-pentynoic acid derivative (**103**) (Scheme 30) was synthesized because it is similar to *N*-acylglycinate and therefore it might have a better chance to pass through the plasma membrane. To obtain this compound, pentynoic acid (**101**) was treated with benzyl glycinate (*p*-TsOH salt) in the presence of diphenylphosphoryl azide (DPPA) and

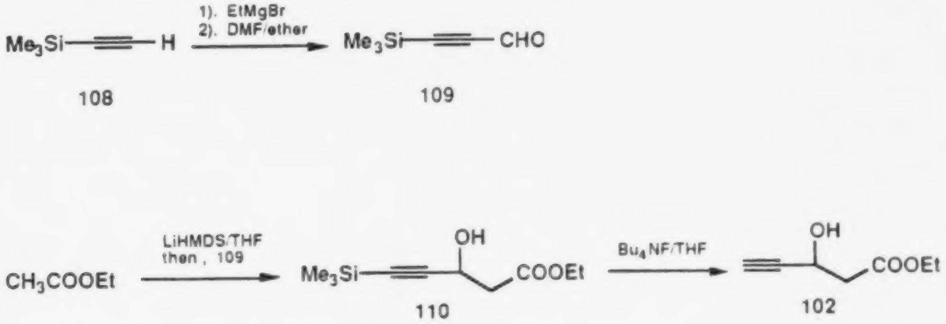
Scheme 30.


COOH
101
COOBn
NH2 p-TsOH
DPPA, DMF
0 °C
H
N
COOBn
O
107
NaOH
THF/H2O
H
N
COOH
O
103

triethylamine to give **107** (94%),[182] which was then selectively hydrolyzed to remove the benzyl group to afford **103** in 15% yield.

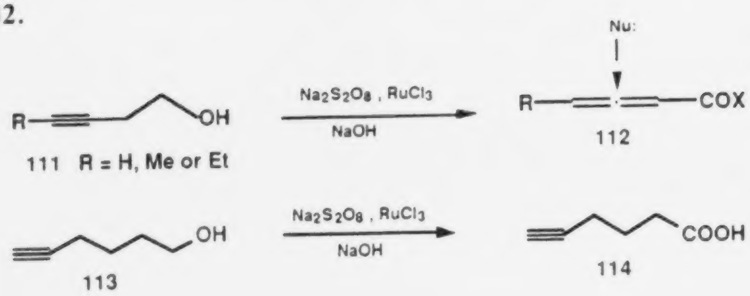
Ethyl 3-hydroxy-4-pentynoate (**102**) was synthesized by Dr. Fionna M. Martin of our group (Scheme 31). Trimethylsilylacetylene (**108**) was first treated with ethyl magnesium bromide, and the resulting intermediate was quenched with DMF to give the acetylene aldehyde **109** (80%).[183] Aldol-type condensation of **109** with the enolate derived from the reaction of ethyl acetate with LiHMDS afforded the silyl protected alkynoate **110** (77%).[110] Deprotection[184] of the trimethylsilyl group by tetrabutylammonium fluoride in THF gave the desired ethyl 3-hydroxy-4-pentynoate (**102**) in 77% yield.

Scheme 31.



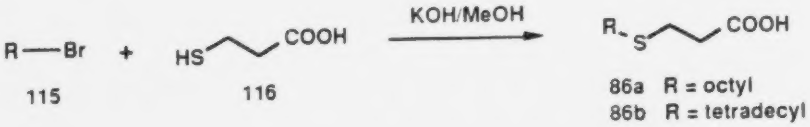
The syntheses of 3-alkynoic acids (**84**) from direct oxidation of the corresponding 3-alkynols proved to be troublesome. Several commercially available 3-alkynols (**111**) (R = H, Me, Et) (Scheme 32) were treated with sodium persulfate and catalytic amounts of ruthenium trichloride in basic solution according to the literature procedure.[199] All the reactions failed to give the desired products, but instead produced complex mixtures of unidentified compounds. The allenic intermediates (**112**) probably form in these reactions and react with nucleophiles (e.g., hydroxide). In contrast, 5-hexynol (**113**) was easily transformed into the 5-hexynoic acid (**114**) (Scheme 32) using the same method.[185]

Scheme 32.


Nu:
R
OH
111 R = H, Me or Et
Na2S2O8 , RuCl3
NaOH
R
COX
112
OH
113
Na2S2O8 , RuCl3
NaOH
COOH
114

Because the alkylthiopropionic derivatives (**86**) were reported to be very potent inhibitors for the acyl-CoA dehydrogenase,[186] they were subsequently synthesized from the reaction of alkyl bromides (**115**) with 3-mercaptopropionic acid (**116**) under basic conditions by Dr. Fionna M. Martin (Scheme 33).[187]

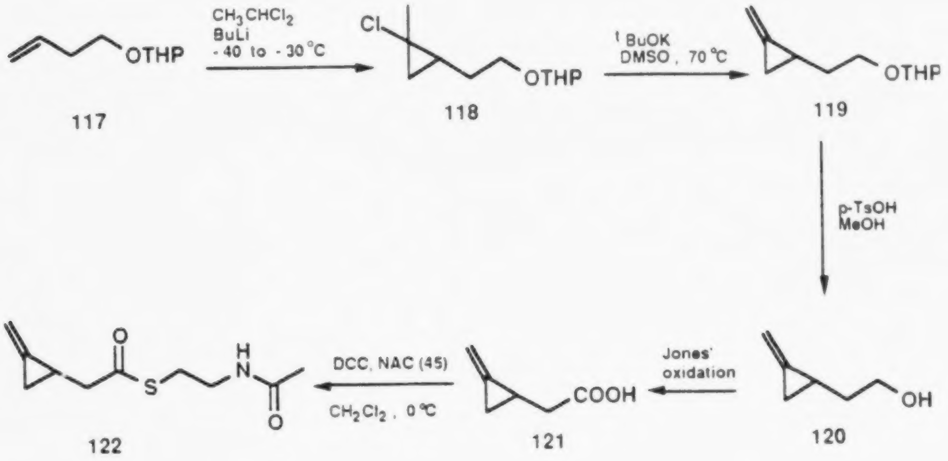
Scheme 33.


R—Br
115
+
HS
COOH
116
KOH/MeOH
R
S
COOH
86a R = octyl
86b R = tetradecyl

The well-known β-oxidation inhibitor, hypoglycin A (**83**), was isolated from the unripe ackee fruit,[188] which was brought from Jamaica by Dr. Evon Bolessa of our group. Since the active derivative of hypoglycin A (**83**) is MCPA-CoA (**89**), the syntheses of its analogs, methylenecyclopropaneacetic acid (**121**) (MCPA) and the NAC thioester derivative of MCPA (**122**) were undertaken (Scheme 34).[189] The carbenoid generated *in situ* from 1,1-dichloroethane and BuLi added to the double bond of THP ether **117** to afford the cyclopropyl derivative **118** (33%). The starting material **117** was available from the treatment of 3-butenol with 2,3-dihydropyran under acidic conditions (92%). Basic elimination of HCl from **118** produced the methylenecyclopropyl compound **119** (86%). The THP group was removed (quantitative yield) under acidic conditions, and the free alcohol **120** was oxidized by Jones' reagent to the MCPA **121** (78%). Finally,

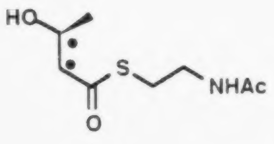
121 was converted to the thioester **122** in 44% yield using the method developed for the synthesis of **34d**.

Scheme 34.

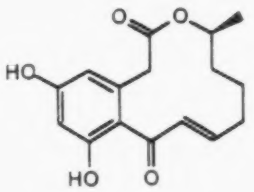

CH3CHCl2
BuLi
-40 to -30 °C
Cl
OTHP
117
118
119
120
121
122
ᵗBuOK
DMSO, 70 °C
p-TsOH
MeOH
Jones' oxidation
COOH
OH
DCC, NAC (45)
CH2Cl2, 0 °C
O
S
H
N

Intact Incorporation of Advanced Precursors into Dehydrocurvularin (3)

With the previously prepared doubly ^{13}C-labeled 3-hydroxybutanoate thioester **34d** and a variety of β-oxidation inhibitors in hand, the feeding experiments with *A. cinerariae* were initiated. However, analysis of the ^{13}C NMR spectra of labeled dehydrocurvularin (**3**) isolated from these fermentations indicated that all the initial experiments failed to give



34d



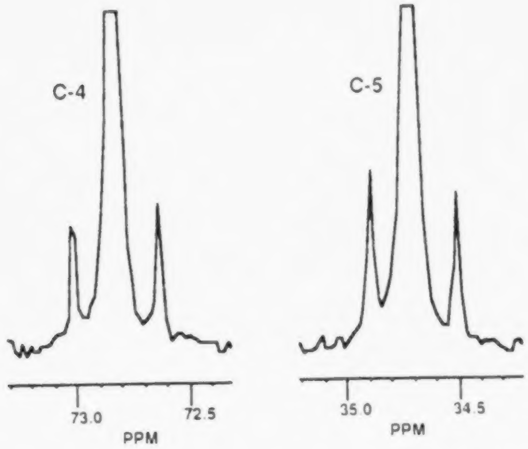
3

intact incorporation of the labeled precursor **34d**, but rather showed the incorporation of labeled acetates, derived from the β-oxidation of **34d**.

It is known that a high concentration of glucose can partially suppress the β-oxidation pathway in mammalian systems because their acetate replacement can be met by the catabolism of glucose.[168] A replacement medium with high glucose was thus used. The precursor **34d** and β-oxidation inhibitor (4-pentynoic acid (**101**), or ethyl 2-bromoacetate (**93**)) were added to the cultures of *A. cinernariae* with the replacement medium. Again, intact incorporation of the labeled precursor was not observed. To overcome this problem, other measures had to be taken to reduce the degradative ability of β-oxidation enzymes.

One approach would involve the use of a mutant of *A. cinernariae* having limited β-oxidation capacity compared to the wild-type organism. A mutant lacking the ability to grow on fatty acids as the sole carbon source may have a low β-oxidation capability. Therefore, mutagenesis of *A. cinerariae* was attempted. A mutant unable to grow well on fatty acid (oleic) culture was generated by Dr. Yuko Yoshizawa of our group and another precursor incorporation experiment was performed. Labeled **34d**, along with the β-oxidation inhibitor, 4-pentynoic acid (**101**), were administered to the replacement medium of the mutant *A. cinernariae*. In the ^{1}H decoupled ^{13}C NMR spectrum of labeled dehydrocurvularin (**3**) isolated from this experiment, two small coupled signals were clearly observed for the resonances of C-5 and C-4 (Figure 10). In addition, there were also large singlet peaks due to the natural abundance of ^{13}C and breakdown of **34d** to labeled acetates before incorporation. The coupled ^{13}C-^{13}C doublets in C-4 and C-5 are unequivocal evidence that some **34d** had been incorporated into **3** as an intact unit.

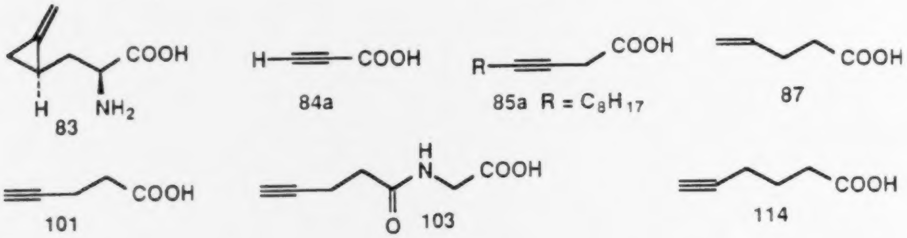
Figure 10. Expansions of ^{1}H-decoupled ^{13}C NMR spectra of **3** after incorporation of **34d**



With the success of this preliminary experiment, other β-oxidation inhibitors were tested in order to improve the intact incorporation rate for the precursor **34d**. The results from these experiments are listed in Table 2.

Intact incorporation was observed in certain cases, and it appears that 4-pentynoic acid (**101**) is the best of the β-oxidation inhibitors tested (Table 2). Interestingly, the best intact incorporation rate (5%) with **101** as inhibitor is less than half that observed by Dr. Yoshizawa (12%) with the same inhibitor. In Dr. Yoshizawa's experiment the precursor **34d** and inhibitor **101** were fed to the mutant *A. cinerariae* in two portions at 12 h intervals starting at 96 h. In the above experiment, **34d** and **101** were added four times at 24 h intervals starting at the same time. It appears that the timing of feeding the precursor (**34d**) and inhibitor (**101**) is crucial for the intact utilization of **34d**. This idea was confirmed by a better intact incorporation (>12%) of **34d** in the presence of **101** with a new feeding protocol (fed four times beginning at 96 h at 8 h intervals).

Table 2. Effect of β-oxidation inhibitors on intact incorporation of **34d** into dehydrocurvularin (**3**).

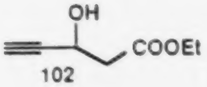


Inhibitor	**83**	**84a**	**85a**	**87**	**101**	**103**	**114**
%Intact	5	<1	5	<1	5	<1	3

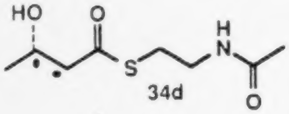
*Inhibitors (total 0.16 mmol) and precursor **34d** (40 mg) were added together at 24 h intervals in 4 equal portions in 98% EtOH (total 1.6 mL) to 96 h cultures of mutant *A. cinerariae* in high glucose replacement medium (125 mL).

**Minimum % intact incorporation is obtained by comparison of the area of coupled signals and the area of the singlet peak. Absolute incorporation rate in each case is 1-2%.

The incorporation of the diketide precursor **34d** into dehydrocurvularin (**3**) by wild type *A. cinerariae* was then explored. Since the precursor **34d** bears a hydroxy group at the β-position, one of the first degradative enzymes to operate on this compound would probably be a β-hydroxyacyl-CoA dehydrogenase, which would oxidize the hydroxyl group to a keto functionality. Based on this idea, it appeared that ethyl 3-hydroxy-4-pentynoate (**102**) might irreversibly inactivate this enzyme through mechanism-based formation of a highly reactive enzyme-bound Michael acceptor, 3-oxo-4-pentynoate.



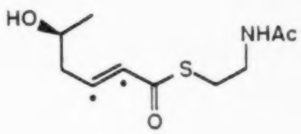
102



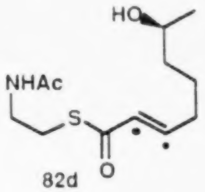
34d

Addition of the precursor **34d**, in the presence of **102** as a β-oxidation inhibitor, to the normal or replacement medium of *A. cinerariae* allows 9% or 14%, respectively, of the labeled diketide (**34d**) to be incorporated intact into dehydrocurvularin (**3**).

As a result of the success of the above experiments with a 4-carbon atom precursor (**34d**), the syntheses and incorporation of larger precursors (e.g., **35d** and **82d**) were undertaken.



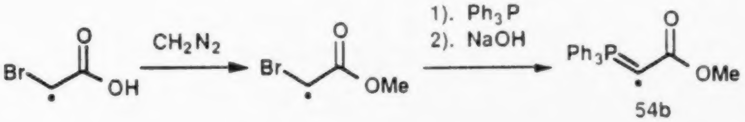
35d



82d

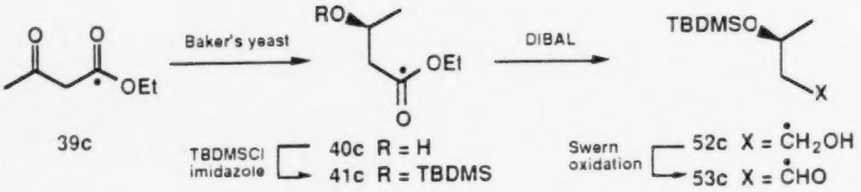
Preparation of the triketide **35d** began with the conversion of [2-^{13}C]bromoacetic acid (isotopic purity 99% ^{13}C) to its methyl ester with diazomethane (quantitative yield) followed by sequential treatments with triphenylphosphine and sodium hydroxide to afford the Wittig reagent **54b** in 86% yield (Scheme 35).[119]

Scheme 35.



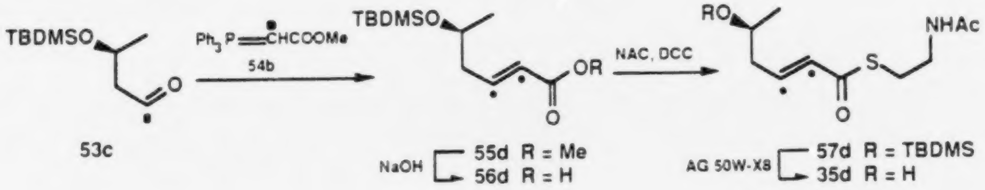
Ethyl [1-^{13}C]acetoacetate (**39c**) was then synthesized using the same procedures developed for the synthesis of **39d**. Thus, ethyl [1-^{13}C]acetate (**38b**), obtained from sodium [1-^{13}C]acetate (isotopic purity 99% ^{13}C) and triethylphosphate, is coupled with acetyl chloride to afford **39c** (62%).[109,110] Baker's yeast reduction (50%) gives **40c**,[112] which is then treated with TBDMS chloride (99%) to give the silyl ether ester (**41c**). Reduction (87%) of **41c** by DIBAL according to Seebach's method[111] followed by Swern oxidation (79%)[117] produced the [1-^{13}C]-aldehyde **52c** (Scheme 36).

Scheme 36.


39c
Baker's yeast
RO
OEt
TBDMSCl imidazole
40c R = H
41c R = TBDMS
DIBAL
TBDMSO
X
Swern oxidation
52c X = CH₂OH
53c X = CHO

Condensation of the aldehyde **53c** with **54b** gives the α,β-unsaturated ester **55d** (61%) as an inseparable mixture of *Z* and *E* isomers (*Z*/*E* = 4.5/95.5), as determined by ^{13}C NMR spectrometry. Hydrolysis (95%) of this mixture with sodium hydroxide by a literature method[111] followed by esterification (93%) of the acid **56d** with *N*-acetylcysteamine (NAC)[120] leads to the NAC thioester **57d** (Scheme 37).

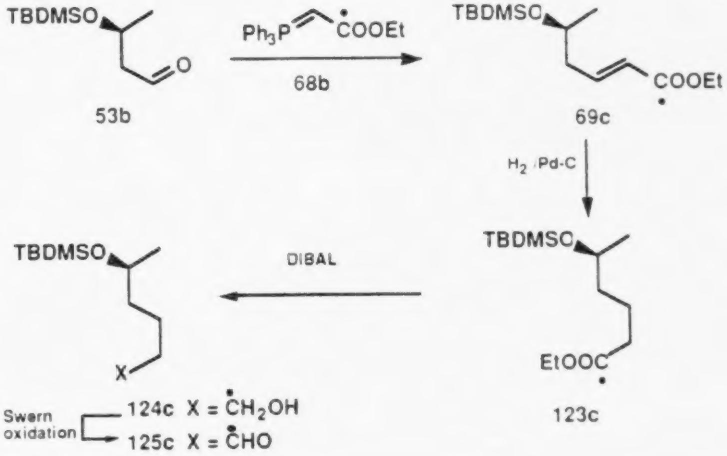
Scheme 37.


TBDMSO
53c
Ph₃P=CHCOOMe
54b
TBDMSO
OR
NaOH
55d R = Me
56d R = H
NAC, DCC
RO
NHAc
S
AG 50W-X8
57d R = TBDMS
35d R = H

Attempts to remove the silyl group of **57d** with boron trifluoride,[115] tetrabutylammonium fluoride,[190] or a hydrogen fluoride-pyridine complex failed.[41b] However, treatment of **57d** with AG 50W-X8 resin in methanol at 40 °C by Corey's procedure[121] cleaves the silyl group to produce the [2,3-^{13}C]-labeled precursor **35d** in 78% yield (isotopic purity 99% $^{13}C_2$; optical purity 90% ee. Determination of the optical purity of **35d** will be discussed after the synthesis of the tetraketide **82d**).

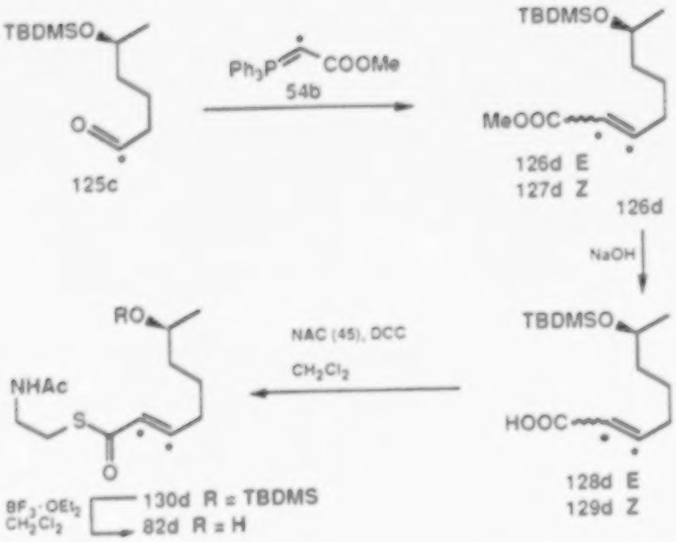
To synthesize the tetraketide precursor **82d**, monolabeled **69c** was produced in 79% yield by reaction of the Wittig reagent **68b** with aldehyde **53b** (Scheme 38), which was obtained from the commercially available (*S*)-1,3-butanediol in the same manner as

Scheme 38.


TBDMSO
O
53b
Ph_3P
COOEt
68b
TBDMSO
COOEt
69c
H_2 /Pd-C
TBDMSO
EtOOC
123c
DIBAL
TBDMSO
X
Swern oxidation
124c X = CH_2OH
125c X = CHO

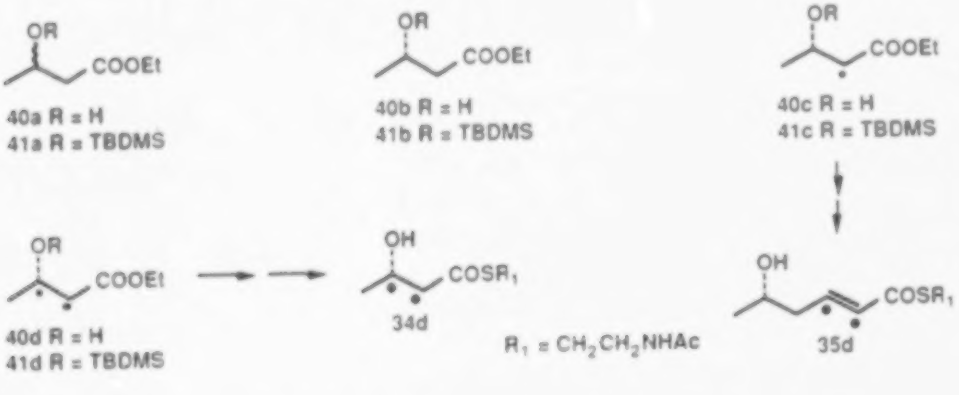
shown earlier in Scheme 17.[111] The α,β-unsaturated ester **69c** was then hydrogenated (95%) to the saturated ester **123c**,[191] reduced by DIBAL (80%), and reoxidized (78%) to the corresponding aldehyde **125c**.[191] The aldehyde **125c** condensed with Wittig reagent **54b** in methanol to give a mixture of *E*-and *Z*-isomers (67/33) (**126d** and **127d**, respectively), in quantitative yield (Scheme 39).[192] The major product **126d** was separated from the minor *Z*-isomer **127d** and converted to the corresponding acid **128d** (quantitative yield) by treatment of sodium hydroxide.[111] Conversion of **128d** to the NAC thioester **130d** (56%) with NAC and DCC, followed by the removal of the silyl protecting group with boron trifluoride etherate,[115] produced **82d** (53%) (along with recovered silyl ether **130d** (47%)) .

Scheme 39.


TBDMSO
O
125c
Ph$_3$P=COOMe
54b
TBDMSO
MeOOC
126d E
127d Z
126d
NaOH
TBDMSO
HOOC
128d E
129d Z
NAC (45), DCC
CH$_2$Cl$_2$
RO
NHAc
S
O
BF$_3$·OEt$_2$
CH$_2$Cl$_2$
130d R = TBDMS
82d R = H

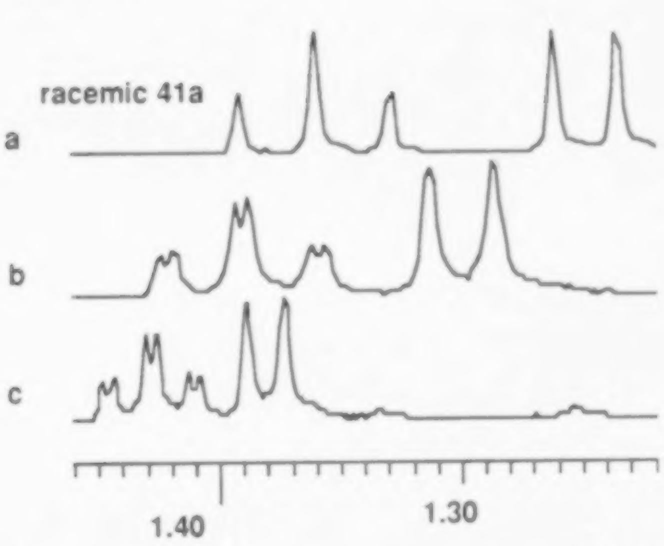
The *Z*-isomer **127d** is more difficult to hydrolyze than the *E*-isomer **126d**. All attempts to obtain the NAC thioester of the *Z*-isomer failed since the reaction of the *Z*-acid **129d** with NAC resulted in isomerization to the *E*-isomer **130d**.

The optical purities of the precursors **34d**, **35d**, and **82d** were determined using the optically active NMR shift reagent, tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato], europium (III) derivative [Eu(hfc)$_3$].[195] To determine the optical purities of **34d** and **35d** (both derived from the silyl ether ester (**41d** and **41c**)), the unlabeled


OR
COOEt
40a R = H
41a R = TBDMS
OR
COOEt
40b R = H
41b R = TBDMS
OR
COOEt
40c R = H
41c R = TBDMS
OR
COOEt
40d R = H
41d R = TBDMS
OH
COSR$_1$
34d
R$_1$ = CH$_2$CH$_2$NHAc
OH
COSR$_1$
35d

racemic material **41a** was treated with the NMR shift reagent. Several peaks in the ^{1}H NMR spectrum then separated (e.g., the CH_3 of the ethyl group, Figure 11). When the optically active labeled **41c** (for **35d**), **41d** (for **34d**) and unlabeled **41b** were treated with the reagent, only a single isomer could be detected in the corresponding 400 MHz ^{1}H NMR spectra. Subsequently, the unlabeled **41b** from both yeast reduction of acetoacetate and from the lipase catalyzed resolution of the racemic material (**41a**) were mixed with known amounts of racemic **41a**, and the expected peak separations in the corresponding ^{1}H NMR spectra were observed upon the addition of the NMR shift reagent. Since the peaks are not well resolved, the calculated enantiomeric excess for **41a** from yeast reduction (95%) and from the lipase resolution (99%) may not be totally accurate. However, judging by the optical rotations of the corresponding ethyl (*S*)-hydroxybutyrate (**40a**) (+38.7° (yeast-reduction) and +41.9° (lipase-resolution); for literature: +37.2° for

Figure 11. Expansions of ^{1}H NMR spectra of **41a** after addition of optically active shift reagent Eu(hfc)$_3$. The amounts of Eu(hfc)$_3$ added to 3 mg of **41a** in 0.4 mL $CDCl_3$ were: a) 0 mg; b) 1.5 mg; and c) 2.1 mg)



85% ee[196] and +41.6° for 96.7% ee[197]) both compounds appear to have at least 90% ee and 95% ee, respectively. Labeled compounds **41c** and **41d** were also treated with the NMR shift reagent, but no expected peak separation was observed. Therefore, they should have similar optical activity (90% ee) to the unlabeled material (from yeast reduction). The precursors **34d** and **35d**, derived from **41d** and **41c**, respectively, should posses an enantiomeric excess of approximately 90%.

The precursor **82d** is derived from **51b**; treatment of **51b** with the NMR shift reagent Eu(hfc)$_3$ produced splitting of the C-4 methyl signal and this result was confirmed by the same peak separation on the ^{1}H NMR spectrum of the racemic material **51a** (Figure 12). The enantiomeric excess for **82d** was found to be 70%.

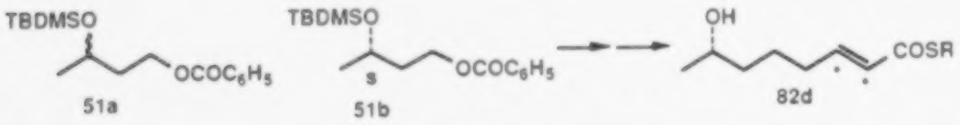
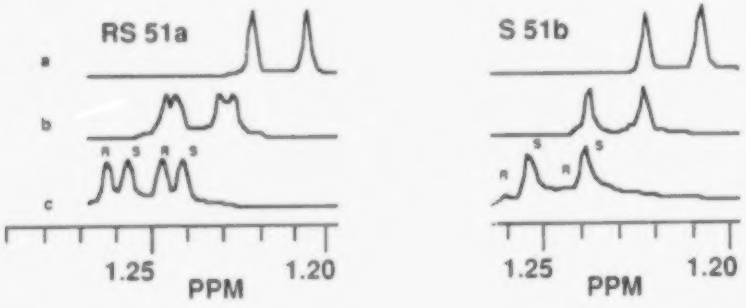
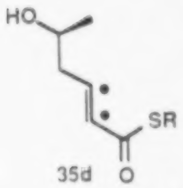
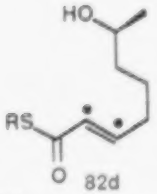


Figure 12. Expansions of ^{1}H NMR spectra of **51** after addition of optically active shift reagent Eu(hfc)$_3$. The amounts of Eu(hfc)$_3$ added to 3 mg of **51** in 0.4 mL CDCl$_3$ were: a) 0 mg; b) 0.9 mg; and c) 1.5 mg. Since **51b** is derived from commercially available (3*S*)-1,3-butanediol , the major peaks (upfield) on spectrum c correspond to the *S*-isomer.

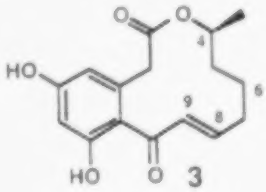


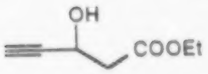
The triketide derivative **35d** and the tetraketide derivative **82d** were then administered to cultures of *A. cinerariae*. The additions were similar to the previous method, but were done using a combination of three β-oxidation inhibitors, **102**, **86a** and **86b**, in an attempt to rapidly obtain intact incorporation of these relatively large precursors.

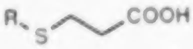

35d


82d

R = $AcNHCH_2CH_2$

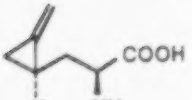

3


102

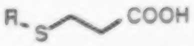

86a R = $Me(CH_2)_7$
86b R = $Me(CH_2)_{13}$

Although the isolated yield of dehydrocurvularin (**3**) was low, ^{13}C NMR analyses of the labeled **3** from these experiments indicated greater than 50% intact incorporation of the tetraketide **82d** into **3**. However, intact incorporation of triketide **35d** was not observed. To determine which of the three inhibitors was most effective in assisting the intact incorporation of **82d**, further experiments with **82d** employed each of the three compounds individually as well as hypoglycin (**83**). The results (Table 3) indicate that the tetradecyl-3-thiopropionic acid (**86b**) is remarkably effective at enhancing intact utilization of **82d**. Although the yield of dehydrocurvularin (**3**) is low (1/3 of the normal amount), there is little if any degradation of the tetraketide precursor. This is evident from the coupled resonances in the ^{13}C NMR spectrum of **3** at C-8 and C-9 (Figure 13), and also from the high recovery of the unutilized precursor **82d** (RP-HLPC).

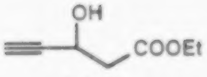
Table 3. Effect of β-oxidation inhibitors on intact incorporation of **82d** into dehydrocurvularin (**3**).



83



86a R = Me$(CH_2)_7$
86b R = Me$(CH_2)_{13}$

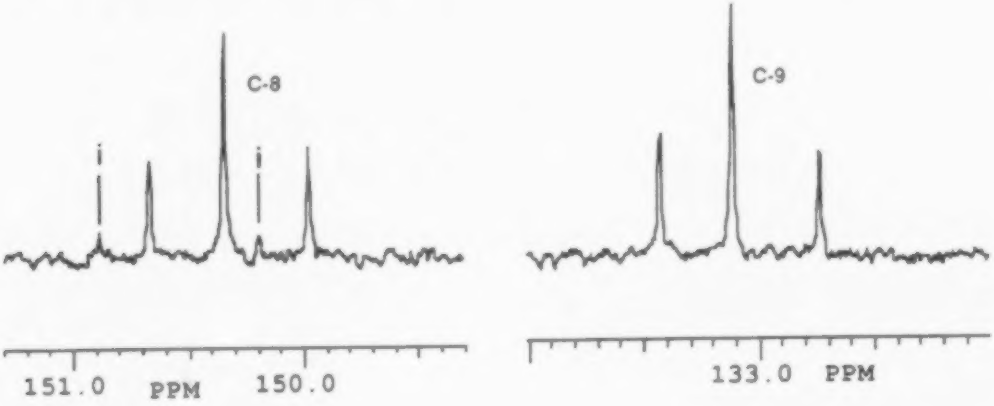


102

Inhibitor	no	**83**	**86a**	**86b**	**102**	**86a, 86b and 102**
% Intact	7	7	7	70	16	50

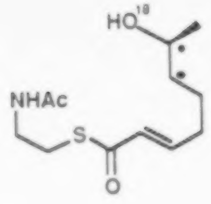
*For **86a**, there was little production of **3**, 7% is an estimated number.

Figure 13. Expansions of ^{1}H-decoupled ^{13}C NMR spectra of **3** after incorporation of **82d** (i signals due to contamination by **82d**)

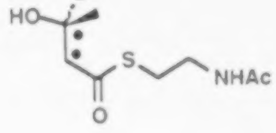


In order to confirm that the other end of the precursor was not undergoing an unexpected degradation, the NAC thioester of (*S*)-[2,3-$^{13}C_2$, 7-*hydroxy*-^{18}O]-7-hydroxyoct-2-enoate

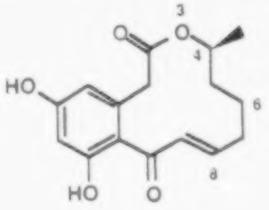
(**82f**) was synthesized. Incorporation of this precursor would confirm that the oxygen atom attached to C-4 of **3** originates from the tetraketide.


HO^{18}
NHAc
S
O

82f



34e

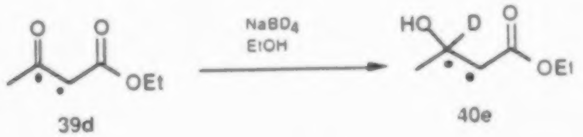

3
4
6
8
HO
HO
O
O

3

The same reasoning suggested that the triply labeled precursor (**34e**) could help detect any unexpected changes in the oxidation state with the diketide precursor.

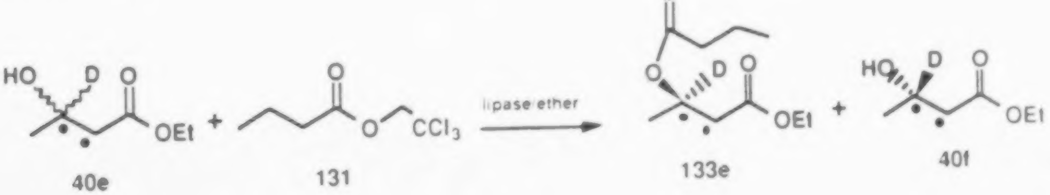
To obtain the key intermediate for the preparation of **34e**, ethyl [2,3-^{13}C, 3-^{2}H]-3-hydroxybutyrate (**40e**), was generated by sodium borodeuteride ($^{2}H_4$, 99%) reduction of ethyl [2,3-^{13}C]acetoacetate (**39d**) (97% yield, Scheme 40). The resulting racemic mixture

Scheme 40.


O
O
OEt
39d
NaBD$_4$
EtOH
HO
D
O
OEt
40e

40e was resolved by porcine pancreatic lipase (PPL) catalyzed transesterification with trichloroethyl butyrate (**131**) (Scheme 41).[193] Separation of the desired *S*-isomer **40f**

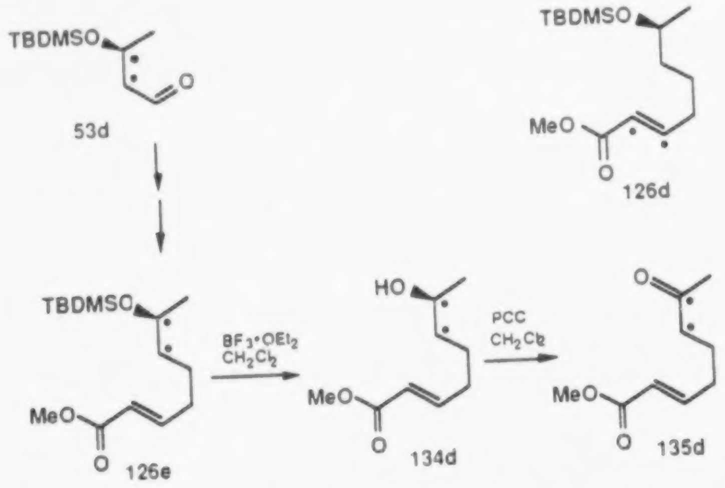
Scheme 41.


HO
D
O
OEt
40e
+
O
O
CCl$_3$
131
lipase ether
O
O
D
O
OEt
133e
+
HO
D
O
OEt
40f

(16%) from the reaction mixture proved to be difficult, but in a preliminary experiment unlabeled ethyl (*S*)-3-hydroxybutyrate (**40a**) was isolated in 32% yield with over 95% ee. Thus, the target compound **34e** (isotopic purity 99% $^{13}C_2$, 99% $^{2}H_2$; optical purity 95% ee) (13% overall yield from **40f**) was prepared from **40f** by a procedure analogous that used to synthesize **34d** (Scheme 16). Unfortunately, the product **40f** was contaminated with 15% trichloroethanol which proved troublesome to remove from the small amount of labeled material.

Introducing an oxygen-18 atom into the molecule is the key step for the synthesis of the tetraketide **82f**. Since ketones undergo oxygen exchange rapidly in water, preparation of the keto compound **135d** was targeted (Scheme 42). The silyl ether **126e**, obtained

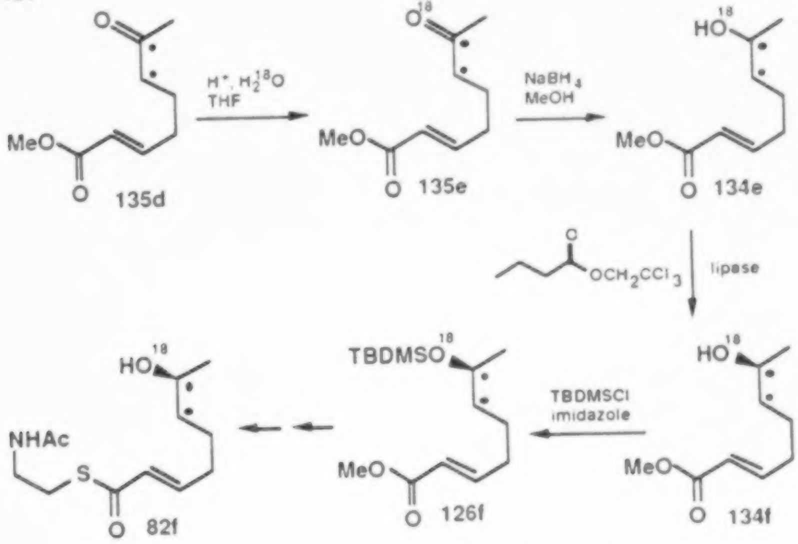
Scheme 42.



from [2,3-^{13}C]-3-*tert*-butyldimethylsiloxybutanal (**53d**) using methods similar to those for the preparation of **126d**, reacts with boron trifluoride etherate to give the hydroxy compound **134d** in 87% yield (with 12% recovery of **126e**). The oxidation of **134d** with pyridinium chlorochromate (PCC)^{41b} gives the desired keto compound **135d** in 90%

yield. The ketone oxygen of **135d** exchanges with ^{18}O-water under acidic conditions,[194] to give the ^{18}O labeled keto compound **135e** (Scheme 43). This compound is immediately

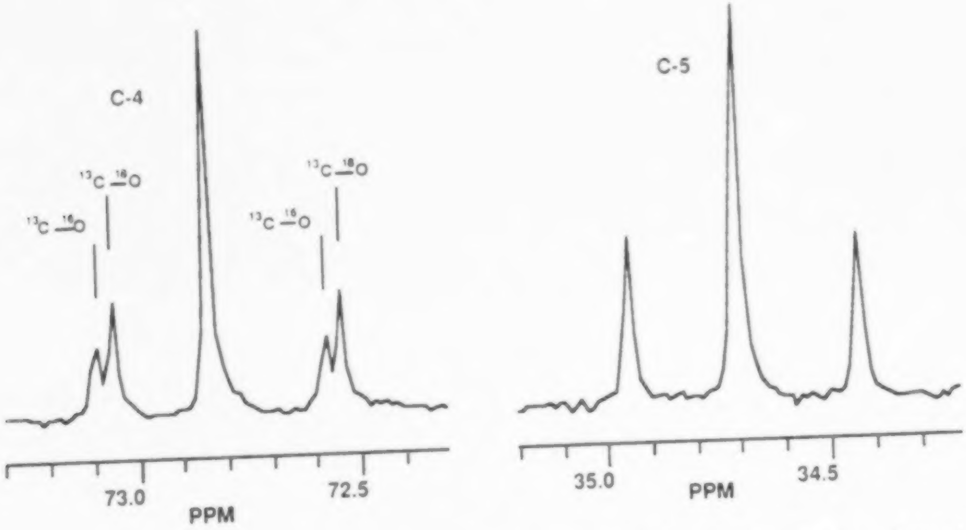
Scheme 43.


135d
H^+, $H_2^{18}O$
THF
135e
NaBH$_4$
MeOH
134e
OCH$_2$CCl$_3$
lipase
134f
TBDMSCl
imidazole
126f
82f
MeO
HO
TBDMSO
NHAc
S
18

reduced to the corresponding hydroxy product **134e** with sodium borohydride in methanol (89%). The oxygen-18 labeled racemic **134e** is then resolved by lipase catalyzed transesterification[193] to afford the *S*-isomer **134f** (49%). This reacts with TBDMS chloride in the presence of imidazole to produce the silyl ether **126f** (72%) (Scheme 43). The desired thioester **82f** (isotopic purity 99% $^{13}C_2$, 66% ^{18}O) is obtained from **126f** in an overall 24% yield using the same strategy developed for the synthesis of its analogue **82d**.

The precursor **82f**, and inhibitor **86b**, were then administered to a culture of wild-type *A. cinerariae* as previously described. A portion of the ^{1}H-decoupled ^{13}C NMR spectrum of the resulting dehydrocurvularin (**3**) (C-5 and C-4) is shown in Figure 14.

Figure 14. Expansions of ^{1}H-decoupled ^{13}C NMR spectra of **3** after incorporation of **82f**. Signals due to ^{13}C-^{18}O have upfield shifts comparing to signals due to ^{13}C-^{16}O.[194]



Integration of the coupled and uncoupled signals for C-5 and C-4 indicates that no less than 70% of the **82f** utilized in the biosynthesis of **3** is incorporated intact. More interestingly, all the oxygen label is retained in **3** within experimental error.

Unfortunately, when the diketide **34e** was added to the culture of *A. cinerariae*, no intact incorporation was observed. This is probably due to the fact that, because of the small amount of the material, **34e** was added in approximately one-seventh of the normal amount.

CONCLUSIONS

The results from the biosynthetic studies on fungichromin (**2**) suggest that new modified polyene antibiotics, such as isochainin (**19**), 14-hydroxyisochainin (**20**), 1'-hydroxyisochainin (**21**), and 1',14-dihydroxyisochainin (**22**), can be produced by *S. cellulosae* through the incorporation of oleate analogues (e.g. **6**). Such an approach, incorporation of unusual starter or terminater units into polyketides, potentially could be applied to other systems and allow production of new antibiotics.

The results from the biosynthetic studies on dehydrocurvularin (**3**) indicate that NAC thioesters of functionalized diketide and tetraketide precursors can be incorporated into *acetate-derived* polyketides such as dehydrocurvularin (**3**) by wild-type fungal cultures under appropriate conditions. Key features for the success of such experiments appear to be: 1) timing of precursor addition; 2) use of replacement media for the incorporation; and 3) simultaneous addition of β-oxidation inhibitors to suppress precursor degradation. Although it is likely that different inhibitors may be suitable for particular precursors, in the present work ethyl 3-hydroxy-4-pentynoate (**102**) and 3-tetradecylthiopropanoic acid (**86b**) proved especially effective. The reasons for the failure of intact incorporation of triketide **35d** into dehydrocurvularin (**3**) are unknown, but the possibility exists that the stereochemistry of the double-bond in the precursor should be *cis* instead of *trans*. Alternatively, the polyketide synthase machinery may permit loading of external precursors only at certain chain lengths or oxidation states. Such experiments, in addition to confirming the structures of hypothetical enzyme-bound intermediates, promise to allow incorporation of altered precursor analogues for biosynthetic generation of new polyketide antibiotics.

EXPERIMENTAL

General

All non-aqueous reactions were performed in oven-dried glassware under a positive pressure of argon. All solvents were distilled. Solvents for anhydrous reactions were dried according to Perrin *et al.*[198] All reagents employed were of American Chemical Society (ACS) grade or finer. Air sensitive reagents were handled under an atmosphere of dry Ar. Commercial organometallic reagents were obtained from Aldrich Chemical Co. *n*-Butyllithium solution was periodically titrated against menthol/phenanthroline. Amino acids and amino acid derivatives used as starting materials were obtained from Sigma Chemical Co. Porcine pancreatic lipase was obtained from Sigma and stored at 0 °C. Freeze-dried specimens of *Streptomyces cellulosae* ATCC 12625 and *Alternaria Cinerariae* ATCC 11784 were purchased from American Type Culture Collection (ATCC) (Rockville, Maryland). All commercially available labeled compounds were purchased from Cambridge Isotope Laboratories (Woburn, MA). The removal of solvents refers to evaporation *in vacuo* on a rotary evaporator followed by evacuation to constant sample weight (<0.05 mm Hg). All reactions were followed by thin layer chromatography (TLC) and visualized using UV fluorescence, iodine staining, dodecamolybdophosphoric acid, and/or ninhydrin spray (for amino acids). Commercial thin layer and preparative layer chromatography plates were: normal silica (Merck 60 F-254) or reverse-phase (Merck RP-8 F-254S). Silica gel for column chromatography was Merck type 60, 70-230 mesh. Flash chromatography was performed according to Still *et al.* using Merck type 60, 230-420 mesh silica gel.[199] Normal phase medium pressure liquid chromatography (MPLC) used a column of Merck Kieselgel 60 H (*ca.* 55 g, 2.5 x 30 cm). Reverse phase MPLC was performed on Merck Lobar Lichroprep RP-8 column, size B. All solvent mixtures are listed as volume ratios, and all medium pressure liquid chromatography was performed using solvents which were previously degassed under vacuum. The cation exchange resin used was Bio-Rad AG 50, 50 x 8 (H^+ form, 50-100 mesh). Water was obtained from a Milli-Q reagent water system.

High pressure liquid chromatography (HPLC) was performed on either Hewlett Packard 1082B, or Beckman System Gold instruments with variable wavelength UV detector set at 357 nm (for polyenes) or 293 nm (for dehydrocurvularin). Columns were Waters μ-Bondapak Radial-Pak cartridges (two reverse phase Radial-Pak C_{18} columns, 1 x 10 and 2.5 x 100) used with a Waters Z-module compression unit. HPLC grade acetonitrile (190 nm cutoff) and methanol were obtained from Terochem (Edmonton, AB). All HPLC solvents were prepared fresh daily and filtered with a Millipore filtration system under vacuum before use.

Gas chromatography (GC) was performed on a Hewlett Parkard 5890A instrument fitted with a RSL-300 (10 M x 0.53 bonded FSOT) column (Alltech) with helium as the carrier gas. Compounds were detected using a flame ionization detector (FID). Temperatures for kugelrohr distillation were those of the air bath surrounding the distillation flask, and did not necessarily represent true boiling points (bp).

All literature compounds had IR, ^{1}H NMR, and mass spectra consistent with the assigned structures. Melting points are uncorrected and were determined on a Thomas Hoover or Buchi oil immersion apparatus using open capillary tubes. Optical rotations were measured on Perkin Elmer 241 or 141 polarimeters with a microcell (100 mm, 1 mL). All specific rotations reported were measured at the sodium D line. Ultraviolet (UV) spectra were determined on a Cary 210 or a Pye Unicam SP1700 instrument. Infrared spectra (IR) were recorded on a Nicolet 7199 FT-IR spectrometer. Mass spectra (MS) were recorded at an ionizing voltage of 70 eV on an AEI MS-50 instrument for high resolution electron impact (EI) ionization and on an MS-12 instrument for low resolution EI and for ammonia and isobutane chemical ionization (CI). Fast atom bombardment mass spectra (FABMS) were recorded on an MS-9 spectrometer. Microanalyses were obtained using a Perkin Elmer 240 CHN analyzer.

Nuclear magnetic resonance (NMR) spectra were measured on Bruker WH-200, AM-300, WM-360, WH-400, or Varian 500 instruments in the specified solvent with tetramethylsilane (TMS) as internal standard for ^{1}H NMR. For ^{13}C NMR spectra, the deuterated solvent peak was used as the reference with its position set relative to TMS.

Radioactivity was determined using standard liquid scintillation procedures in plastic 10 mL scintillation vials (Terochem) with Amersham ACS liquid scintillation cocktail. The instruments used were a Beckman LS100C and Beckman 1801. With the Beckman 1801, the automatic quench control was employed to directly determine decompositions per minute (dpm) in single and dual label samples by comparison against a quench curve prepared from Beckman ^{3}H and ^{14}C quenched standards. This automatically calculates ^{3}H/^{14}C ratios but the results were confirmed by analyzing random samples with the addition of standardized ^{14}C-toluene and ^{3}H-toluene solutions (ICN Radiochemicals). The values obtained always agreed within 5% of those calculated by the instrument. Radioactive tlc plates were analyzed with a Berthold LB2760 tlc-scanner.

NMR Method for Determine the Enantiomeric Excess of 41 and 51. ^{1}H NMR spectra were determined on a Bruker WH-400 instrument. A solution of tris[3-(heptafluoropropyl hydroxymethylene)-d-camphorato], europium (III)[195] in $CDCl_3$ (30.0 mg/500 µL, 60.0 µg/µL) was added in portions to an NMR tube containing compound **41** or **51** (3 mg/0.4mL) until a peak separation could be distinguished in the ^{1}H NMR spectrum. For ethyl 3-(*tert*-butyldimethylsiloxy)butyrate (**41**), peak separation appeared when 25 µL (1.5 mg) of the chiral shift reagent was added. For 3-(*tert*-butyldimethylsiloxy)butyl benzoate (**51**) (3 mg/0.4 mL), the peak separation started when 15 µL (0.9 mg) of the chiral shift reagent was added.

General Procedure for Growth of *Streptomyces Cellulosae*, and Isolation of Fungichromin (2).[60] One freeze-dried specimen of *Streptomyces* *cellulosae* (ATCC 12625) was soaked in H_2O (1 mL) for 5 min, and the resulting spore suspension was added to 10 slants (2-3 drops per slant), prepared from bacto-yeast malt extract agar (19 g) and H_2O (500 mL) which had been sterilized at 121 °C for 20 min. The inoculated slants were incubated at 25 °C for 7 days and then stored at 4 °C until needed. The resulting mycelium from one slant was suspended in H_2O (2 mL), and the suspension was added to 2 Erlenmeyer flasks (500 mL), each containing liquid media (100 mL) prepared from bactopeptone (5 g), DIFCO yeast extract (2.5 g), NaCl (4 g), glucose (10 g) and Span 85 (Sigma, 10 mL) made up to 1 L with H_2O, buffered to pH 7.0 with $NaHCO_3$, and then autoclaved at 121 °C for 20 min. The precultures were incubated in a fermenter at 26 °C and 165 rpm in the dark for 48 h. A 2 mL portion of the resulting suspension was then transferred to each of 10 flasks containing medium prepared as above (100 mL/flask); the flasks were then incubated under the same conditions. After 3-4 days, the contents became yellow, and isotopically labeled precursors were added aseptically in 4 portions at 24 h intervals. At 24 h after the last feeding, the mycelium (ca. 25 g fresh weight) was collected by vacuum filtration. The filtrate was extracted (hexanes 66%/benzene 34%, 2 x 500 mL, then EtOAc, 2 x 500 mL). The mycelium was gently boiled in hexanes 66%/benzene 34% (500 mL, 30 min). The cooled mixture was filtered , and the filter cake was extracted with boiling EtOAc (500 mL, 10 min). The combined EtOAc extracts were concentrated *in vacuo* to afford ca. 1.3 g, of yellow solid, which was taken up in MeOH, and filtered. The filtrate was concentrated *in vacuo*. Column chromatography of the residue on Sephadex LH-60 (MeOH) afforded UV-active fractions which were concentrated *in vacuo*. The residue was taken up in MeOH (5 mL), H_2O (2.7 mL) was added, and the thick precipitate was removed on a centrifuge. Medium pressure liquid chromatography (reverse phase, MeOH 65%/H_2O 35%, 1 mL/min, 6 mL fractions) of the supernatant (5 mL) afforded 20-40 mg of **2** from fractions 35-40 after azeotropic removal of solvent *in vacuo* with EtOH: R_f (silica, $CHCl_3$ 22%/MeOH 22%/EtOAc 45%/H_2O 11%, lower phase) **2**, 0.31; **4a**, 0.38. R_f (RP-8, MeOH 65%/H_2O 35%) **2**, 0.35; **4a**, 0.27; ^{13}C

NMR spectral data is given in Table 1; FAB-MS (glycerol-sulfolane matrix) 693 (MNa), 670 (M).

Fermentation of *S. Cellulosae* with ethyl [11-^{13}C,^{14}C]-Butoxytridec-9-enoate (7b) and 7a. Fermentation of *S. cellulosae* was done in the same manner as above, but oleate analogues **7b** and **7a** were added to the production cultures (66 mg/100 mL). The cultures were extracted at day 8 in the usual fashion to isolate labeled the polyene fractions. No sufficient amount of polyenes was isolated. .

Fermentation of *S. Cellulosae* for Production of ^{13}C/^{14}C Labeled 4. Fermentation of *S. cellulosae* was done in the usual fashion with Span 85; labeled sodium [1-^{13}C]acetate (100 mg), sodium [2-^{13}C]acetate (100 mg), sodium [1-^{14}C]acetate (25 μCi) in 70% EtOH (10 mL) were fed to 10 flasks (100 mL each) in 5 portions at 24 h intervals starting at day 2 together with the P-450 inhibitor ancymidol (**29**) (256 mg) in 70% EtOH (10 mL). The cultures were extracted at day 8 in the usual fashion to isolate labeled **4a** and **4b**. After initial purification on MPLC, the fractions containing **4a** were further purified on HPLC and **4a** (9.51 mg, 3.45 mCi/mole) was isolated. For unlabeled compounds, **4a**: IR (acetone cast) 3360 (br), 1725 (m), 1075 (m) cm^{-1}; ^{1}H NMR (200 MHz, CD_3OD) δ 6.25-6.50 (m, 7 H), 6.02 (m, 2 H), 4.88 (m, 1 H), 3.83-4.25 (m, 8 H), 3.22 (m, 1 H), 2.57 (m, 1 H), 1.73 (s, 3 H), 1.20-1.63 (m, 23 H), 0.92 (t, 3 H, J = 7.0 Hz); ^{13}C NMR data given in Table 4 (below); positive ion FAB MS (glycerol) calcd for $C_{35}H_{58}O_{11}Na$ 677.39, found 677.73 (MNa); **4b**: IR (acetone cast) 3360 (br), 1729 (m), 1077 (m), 1050 (m) cm^{-1}; ^{1}H NMR (200 MHz, CD_3OD) δ 6.30-6.60 (m, 7 H), 6.08 (d, 1 H, J = 11.0 Hz), 5.92 (dd, 1 H, J = 15.0, 6.0 Hz), 4.70 (m, 1 H), 4.19 (dd, 1 H, J = 10.5, 4.4 Hz), 3.97-4.08 (m, 5 H), 3.85 (m, 1 H), 3.30 (m, 1 H), 2.36 (m, 1 H), 1.2-1.8 (m, 27 H), 0.92 (t, 3 H, J = 7.0 Hz); ^{13}C NMR data given in Table 4 (below); positive ion FAB MS (glycerol) calcd for $C_{35}H_{58}O_{10}Na$ 661.39, found 661.51 (MNa);

Table 4. ^{13}C Chemical shifts for fungichromin (**2**), filipin III (**4a**), and filipin II (**4b**)

carbon	^{13}C δ^{a,b}		
	2	**4a**	**4b**
29	11.74	11.07	11.39
6'	14.38	14.39	14.39
28	17.96	17.99	18.33
5'	23.65	23.67	23.62
3'	26.01	26.03	28.33
4'	32.88	32.91	32.83
2'	36.22	36.20	30.23
12	39.58	41.58	42.50
4	41.38	42.88	42.68
10	44.34	44.17	44.12
6	45.17	45.15	44.85
8	45.33	45.28	45.07
2	60.35	60.26	54.30
13	70.34	67.45	67.46
11	71.45	71.12	70.98
1'	72.59	72.63	30.26
26	73.25	73.24	73.15
3	73.41	73.63	73.31
7	73.92	73.66	73.39
5	74.08	73.73	73.58
9	74.20	73.96	74.13
27	75.25	75.10	74.45
14	78.31	45.28	45.16

15	80.43	75.83	75.63
18	129.06	128.35	128.07
17	129.91	129.31	129.56
24	131.97	132.20	132.38
22	133.66	133.82	133.65
20	134.13	134.14	134.17
23	134.21	134.29	134.22
25	134.28	134.37	134.45
21	134.81	134.60	134.58
19	135.36	134.97	134.71
16	138.55	140.35	140.60
1	172.98	172.98	175.40

[a] 100.6 MHz ^{13}C NMR spectrum in methanol-d_4 with solvent reference at 49.00 ppm.

[b] For details of spectral assignment of fungichromin (**2**) see ref. 60.

Fermentation of *S. Cellulosae* for Conversion of 4a to 2. Fermentation of *S. cellulosae* was done in the usual fashion with Span 85; the $^{14}C/^{13}C$ labeled **4a** (4.75 mg, 5.53×10^4 dpm, 3.45 mCi/mole) in 98% EtOH (10 mL) was fed to 2 flasks (100 mL each) in 5 portions at 24 h intervals starting at day 2. The cultures were extracted at day 8 as usual to isolate **2**, **4a**, and **4b**. After initial purification on MPLC, the fractions containing **2**, **4a**, and **4b** were further purified on HPLC:

The specific activity of fungichromin (**2**)

solvents MeOH/H_2O	retention time (min)	specific activity (μCi/mole)
62 : 38	32.4	82.6
60 : 40	34.5	73.8
58 : 42	49.2	75.3
56 : 44	55.5	74.8

*HPLC conditions: Waters prepak 25 mm x 10 cm C-18 column, flow rate = 4 mL/min.

Fermentation of *S. Cellulosae* for Incorporation of 34d and 35e. Fermentation of *S. cellulosae* was done in the usual fashion with Span 85; the labeled precursor was fed to the production culture (100 mL each) in equal portions to each flask every 24 h for 5 days beginning at 2 days (For **34d** a total of 102 mg was fed to 5 flasks; for **35e** a total of 20 mg was fed to 4 flasks).

Fermentation of *Alternaria Cinerariae* to Produce Dehydrocurvularin (3). The cultures of *A. cinerariae* ATCC 11784 were maintained on slants composed of Difico potato dextrose agar (39 g/L). Spore suspensions from these were used to inoculate media containing Difico potato dextrose broth (24 g/L). The inoculated flasks (125 mL medium per 500 mL flask) were placed on a rotary fermenter incubating at 26 °C (165 rpm) for 7 days. The cultures were filtered and the filtrate was extracted with EtOAc (3 x 100 mL/125 mL of filtrate) to give, after concentration *in vacuo*, a yellow gum (ca. 200 mg), which was chromatographed on silica (15% EtOAc in $CHCl_3$) to give dehydrocurvularin (**3**) (yield 15-40 mg/125 mL). IR (KBr) 3428 (br), 1712 (s), 1636 (m) cm^{-1}; ^1H NMR (200 MHz, acetone-d_6) δ 6.75 (m, 1 H), 6.58 (m, 1 H), 6.30 (m, 2 H), 4.70 (m,1 H), 3.80 (m, 2 H), 2.32 (m, 2 H), 1.5-2.2 (m, 4 H), 1.21 (d, 3 H, J = 6.4 Hz); ^{13}C NMR (400 MHz, acetone-d_6) δ 198.11 (C-10), 172.47 (C-2), 165.92 (C-11), 163.51 (C-13), 150.35 (C-8), 139.63 (C-15), 133.11 (C-9), 116.21 (C-16), 113.90 (C-14), 103.16 (C-12), 73.09 (C-4), 43.59 (C-1), 34.83 (C-5), 33.15 (C-7), 24.90 (C-6), 20.23 (4-Me); MS (EI) calcd for $C_{16}H_{18}O_5$ 290.1157, found 290.1158 (M);

Fermentation of Mutant *A. Cinerariae* for Incorporation Experiments. A mutant isolated by Dr. Yuko Yoshizawa was used. Fermentations employed the same conditions as above except that after 96 h, the mycelia from two flasks (ca. 10 g) were filtered and washed with replacement medium consisting of: glucose (100 g); Na_2HPO_4 (1 g); $MgSO_4 \cdot 7H_2O$ (0.5 g); KCl (0.5 g); $FeSO_4 \cdot 7H_2O$ (0.01 g) per liter. The mycelia were transferred to a 500-mL Erlenmeyer flask containing 125 mL of the replacement medium. Labeled precursor (**34d** 40 mg;) in 0.8 mL of 98% EtOH together with β-oxidation inhibitor in 0.8 mL of 98% EtOH were added equally in 4 portions at 8 h intervals. Incubation on a rotary shaker at 25 °C for 96 h followed by extraction and purification procedures described above gave **3**.

Fermentation of Wild Type *A. Cinerariae* for Incorporation Experiments. The procedure outlined above for the mutant was followed. Labeled precursor (for **34d**, 40 mg; **35d**, 20 mg; **82d** and **82f**, 15 mg) in 0.8 mL of 98% EtOH and β-oxidation inhibitor (for **102**, 36 mg; for **86b**, 15 mg) in 0.8 mL of 98% EtOH were added equally in 4 portions at 8 h intervals. Extraction and purification as described above gave dehydrocurvularin (**3**).

Ethyl (Z)-16-Phenylhexadec-9-enoate (6). A modification the method of Anderson and coworkers' was followed.[87] Triphenylphosphine (3.30 g, 12.6 mmol) and **17** (3.60 g, 11.5 mmol) in toluene (20 mL) were heated to reflux for 12 h. The mixture was cooled to 20 °C, most of the toluene was removed by syringe, DMF (100 mL) was added, and the solution was cooled to -60 °C. To this was added a solution of $LiN(SiMe_3)_2$ prepared by adding butyllithium (7.00 mL, 1.40 M in hexane) to hexamethyldisilazane (2.34 mL, 11.0 mmol) in THF (6 mL) at -78 °C. The ylide solution was treated with aldehyde **13** (1.73 g, 9.10 mmol) in DMF (20 mL), stirred 1 h at -60 °C, and then warmed to 20 °C. Acetic acid (1 N, 6 mL) was added followed by water (100 mL). The product was extracted into hexane (3 x 150 mL), dried (Na_2SO_4), and concentrated *in vacuo*. The residue was separated by column chromatography (silica gel) using hexane/EtOAc (98/2) to give **6** (1.65 g, 51%). IR ($CHCl_3$ cast) 2930 (m), 2855 (m), 1737 (s), 1653 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.26 (m, 2 H, Ar$\underline{H}$), 7.17 (m, 3 H, Ar$\underline{H}$), 5.34 (t, 2 H, *J* = 4.6 Hz, C$\underline{H}$=C$\underline{H}$), 4.11 (q, 2 H, *J* = 7.1 Hz, OC$\underline{H}_2$), 2.60 (t, 2 H, *J* = 7.8 Hz, PhC$\underline{H}_2$), 2.28 (t, 2 H, *J* = 7.6 Hz, C$\underline{H}_2$COOEt), 1.23 (3 H, t, *J* = 7.1 Hz, OCH$_2$C$\underline{H}_3$), 2.00 (m, 4 H, 2 x C$\underline{H}_2$), 1.60 (m, 4 H, 2 x C$\underline{H}_2$), 1.3 (m, 14 H, 7 x C$\underline{H}_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.82 ($\underline{C}$OOEt), 142.83, 129.82 (2 x C), 128.34, 128.16, 125.51, 60.09 (O$\underline{C}$H$_2$), 35.94, 34.34, 31.44, 29.64, 29.17, 29.14, 29.07, 27.14, 24.94, 14.22 ($\underline{C}$H$_3$); MS (EI) calcd for $C_{24}H_{38}O_2$ 358.2872, found 358.2919 (M); Anal. Calcd for $C_{24}H_{38}O_2$: C, 80.39; H, 10.68. Found: C 80.81; H 10.70.

5-Phenylpentyl *p*-Toluenesulfonate (10). A solution of 5-phenylpentanol (24.3 g, 148 mmol) in CH_2Cl_2 (200 mL) and pyridine (15.8 g, 200 mmol) at 0 °C was treated with *p*-toluenesulfonyl chloride (31.1 g, 163 mmol), and stirred for 12 h at 20 °C. The solution was concentrated *in vacuo*, the residue was dissolved in hexane-EtOAc (600 mL, 9:1), and the resulting solution was cooled to -78 °C and filtered to give **10** (41.7 g, 88%). IR ($CHCl_3$ cast) 1598 (s), 1359 (m), 1177 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.80 (m, 2 H, $Ar\underline{H}$), 7.38-7.10 (m, 7 H, $Ar\underline{H}$), 4.03 (t, 2 H, *J* = 6.8 Hz, $C\underline{H}_2OTs$), 2.55 (t, 2 H, *J* = 8.0 Hz, $PhC\underline{H}_2$), 2.43 (s, 3 H, $OTs\text{-}C\underline{H}_3$), 1.70-1.46 (m, 6 H, 3 x $C\underline{H}_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.55, 142.02, 133.05, 129.70, 128.20, 128.15, 127.72, 125.61, 70.36, 35.50, 30.56, 28.53, 24.84, 21.46; MS (EI) calcd for $C_{18}H_{22}O_3S$ 318.1290, found 318.1275 (M); Anal. Calcd for $C_{18}H_{22}O_3S$: C, 67.89; H, 6.97; S, 10.07. Found: C, 68.15; H, 6.95; S, 10.27.

7-Phenylheptanoic Acid (11). A solution of sodium ethoxide (made by adding sodium (3.31 g, 144 mmol) to ethanol (150 mL)) was added dropwise to a mixture of diethyl malonate (23.1 g, 144 mmol) and **10** (41.7 g, 131 mmol) at 80 °C, and heating at 80 °C was continued for 12 h. The mixture was cooled and a solution of KOH (22.0 g, 392 mmol) in H_2O (150 mL) was added. The solution was heated an additional 3 h at 80 °C, cooled, and then acidified with 6 N HCl and extracted with ether (3 x 200 mL). The dried extracts (Na_2SO_4) were concentrated *in vacuo* and heated at 160 °C for 3 h. Distillation (0.5 mm Hg) at 147-150 °C afforded the known[200] acid **11** (19.0 g, 70%). IR ($CHCl_3$ cast) 3300-2500 (br), 1708 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 10.3 (br s, 1 H, $COO\underline{H}$), 7.32-7.12 (m, 5 H, $Ar\underline{H}$), 2.61 (t, 2 H, *J* = 7.6 Hz, $C\underline{H}_2COOH$), 2.32 (t, 2 H, *J* = 7.4 Hz, $PhC\underline{H}_2$), 1.70-1.30 (m, 8 H, 4 x $C\underline{H}_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 180.50 ($\underline{C}OOH$), 142.60, 128.35, 128.22, 125.59, 35.82, 34.04, 31.21, 28.84 (2 x C), 24.54; MS (EI) calcd for $C_{13}H_{18}O_2$ 206.1307, found 206.1308 (M).

7-Phenylheptanol (12). Lithium aluminum hydride (5.00 g, 130 mmol) in THF (100 mL) was added dropwise to a solution of **11** (18.9 g, 92.0 mmol) in THF (50 mL) at 0 °C over 1 h. The solution was warmed to 20 °C for 1 h, methanol (30 mL) was added, and the mixture was poured into 1 N HCl (200 mL). This was filtered and extracted with ether (3 x 150 mL). The dried (Na_2SO_4) extracts were concentrated *in vacuo* and distilled (113-116 °C, 0.35 mm Hg) to give the known[201] 7-phenylheptanol (**12**) (15.8 g, 89%). IR ($CHCl_3$ cast) 3540 (br), 1030 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.25-7.13 (m, 2 H, Ar$\underline{H}$), 3.57 (t, 2 H, J = 6.8 Hz, $C\underline{H}_2OH$), 2.58 (t, 2 H, J = 8.0 Hz, $PhC\underline{H}_2$), 2.20 (br s, 1 H, $O\underline{H}$), 1.65-1.32 (m, 10 H, 5 x $C\underline{H}_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.67, 128.25, 128.10, 125.45, 62.67, 35.83, 32.57 (2 x C), 31.30, 29.17, 29.14, 25.57; MS (EI) calcd for $C_{13}H_{20}O$ 192.1514, found 192.1513 (M).

7-Phenylheptanal (13). A solution of oxalyl chloride (20.0 mL, 230 mmol) in CH_2Cl_2 (250 mL) was treated with DMSO (34.0 mL, 440 mmol) at -60 °C. The mixture was stirred 5 min at -60 °C and a solution of 7-phenylheptanol (**12**) (4.37 g, 22.7 mmol) in CH_2Cl_2 (20 mL) was added over 10 min. Stirring was continued for 15 min, triethylamine (70.0 mL, 500 mmol) was added, and the mixture was allowed to warm to 20 °C. This was washed consecutively with water (300 mL), 1 N HCl (300 mL), and 5% Na_2CO_3 (300 mL). The dried ($MgSO_4$) organic phase was concentrated *in vacuo* and distilled (130 °C, 0.5 mm Hg) to afford **13** (1.73 g, 40%). IR ($CHCl_3$ cast) 1726 (s), 1179 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 9.77 (t, 1 H, J = 3.0 Hz, $C\underline{H}O$), 7.33-7.11 (m, 5 H, Ar$\underline{H}$), 2.60 (t, 2 H, J = 8.0 Hz, $PhC\underline{H}_2$), 2.41 (dt, 2 H, J = 7.0, 3.0 Hz, $C\underline{H}_2CHO$), 1.72-1.28 (m, 8 H, 4 x $C\underline{H}_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.75 ($\underline{C}HO$), 142.74, 128.40, 128.30, 125.63, 35.93, 31.37, 29.14, 29.05, 28.51, 25.77; MS (EI) calcd for $C_{13}H_{18}O$ 190.1358, found 190.1356 (M).

9-Bromononanol (14). The method of Kang *et al.* was followed.[80] A mixture of 1,9-nonandiol (99.4 g, 621 mmol) and HBr (48%, 85 mL) in benzene (250 mL) was heated under reflux for 30 h with continuous removal of H_2O (ca. 83 mL). The solvent was removed *in vacuo*, and the residue was recrystallized from hexane at -10 °C to give **14** (90.0 g, 65%). Mp 32-33 °C; IR ($CHCl_3$ cast) 3326 (br), 2966 (s), 2932 (s), 2852 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.64 (t, 2 H, J = 6.7 Hz, $C\underline{H}_2OH$), 3.40 (t, 2 H, J = 6.7 Hz, $BrC\underline{H}_2$), 1.85 (m, 2 H), 1.56 (m, 2 H), 1.47-1.25 (m, 10 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 62.78 ($\underline{C}H_2OH$), 33.91, 32.70, 32.57, 29.27, 29.20, 28.58, 28.03, 25.60; MS (EI) calcd for $C_9H_{17}{}^{79}Br$ 204.0514, found 204.0512 ($M-H_2O$); calcd for $C_9H_{17}{}^{81}Br$ 206.0493, found 206.0491 ($(M+2)-H_2O$).

9-Bromononanoic Acid (15). A solution of sodium meta-periodate (87.7 g, 410 mmol) in water (300 mL) was added to a solution of 9-bromononanol (22.3 g, 100 mmol) in CH_3CN (200 mL) and CCl_4 (200 mL). Ruthenium trichloride trihydrate ($RuCl_3{\cdot}3H_2O$) (580 mg, 2.20 mmol) was added,[84] the mixture was stirred at 20 °C for 2.5 h, and the solution was then extracted with CH_2Cl_2 (3 x 200 mL). The dried extracts (Na_2SO_4) were concentrated *in vacuo*, redissolved in ether (300 mL), filtered through a Celite 545 column (5 x 15 cm), and again concentrated *in vacuo*. The resulting solid was distilled (143 °C, 0.25 mm Hg) to afford the known acid **15** (21.1 g, 89%). Mp 36.0-36.3 °C (lit.[202] mp 36.0-36.5 °C); IR ($CHCl_3$ cast) 3300-2500 (br), 1699 (s) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 9.14-8.94 (br s, 1 H, $COO\underline{H}$), 3.40 (t, 2 H, J = 7.0 Hz, $C\underline{H}_2Br$), 2.35 (t, 2 H, J = 8.0 Hz, $C\underline{H}_2COOH$), 1.80-1.96 (m, 2 H, $C\underline{H}_2$), 1.75-1.24 (m, 10 H, 5 x $C\underline{H}_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 180.86 ($\underline{C}OOH$), 34.43, 34.24, 33.11, 29.37, 29.25, 28.88, 28.42, 24.93; MS (EI) calcd for $C_9H_{17}{}^{79}BrO_2$ 236.0412, found 236.0416 (M); calcd for $C_9H_{17}{}^{81}BrO_2$ 238.0392, found 238.0450 (M+2).

Ethyl 9-Bromononanoate (16). A solution of **15** (21.0 g, 88.6 mmol) in ether (150 mL) was treated with thionyl chloride (12.7 g, 107 mmol) and heated to reflux for 4 h.[86] The cooled solution was concentrated *in vacuo*, redissolved in ether (150 mL), cooled to 0 °C, and treated with excess ethanol (10 mL). The mixture was concentrated *in* *vacuo* and distilled (bp 113 °C, 0.2 mm Hg) to give the known[203] ester **16** (23.0 g, 98%). IR ($CHCl_3$ cast) 2932 (m), 1735 (s), 1180 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.13 (q, 2 H, *J* = 7.2 Hz, $OC\underline{H}_2$), 3.41 (t, 2 H, *J* = 6.9 Hz, $BrC\underline{H}_2$), 2.28 (t, 2 H, *J* = 7.4 Hz, $C\underline{H}_2COOEt$), 1.85-1.62 (m, 4 H, 2 x $C\underline{H}_2$), 1.50-1.20 (m, 8 H, 4 x $C\underline{H}_2$), 1.26 (t, 3 H, *J* = 7.2 Hz, $C\underline{H}_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.16 ($\underline{C}OO$), 59.70 ($O\underline{C}H_2$), 33.91, 33.42, 32.47, 28.74, 28.67, 28.24, 27.76, 24.56, 13.94 ($\underline{C}H_3$); MS (EI) calcd for $C_{11}H_{21}{}^{79}BrO_2$ 264.0725, found 264.0730 (M); calcd for $C_{11}H_{21}{}^{81}BrO_2$ 266.0705, found 266.0709 (M+2).

Ethyl 9-Iodononanoate (17). A mixture of **16** (23.0 g, 86.7 mmol) and sodium iodide (15.6 g, 104 mmol) in 2-butanone (200 mL) was heated to reflux with stirring for 18 h.[86] H_2O (200 mL) was added to the cooled mixture, and the solution was extracted with CH_2Cl_2 (3 x 100 mL). The dried extracts (Na_2SO_4) were concentrated *in vacuo* and distilled (131 °C, 0.2 mm Hg) to give **17** (26.5 g, 97%). IR ($CHCl_3$ cast) 2929 (m), 1735 (s), 1177 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.12 (q, 2 H, *J* = 7.2 Hz, $OC\underline{H}_2$), 3.19 (t, 2 H, *J* = 7.0 Hz, $BrC\underline{H}_2$), 2.29 (t, 2 H, *J* = 7.4 Hz, $C\underline{H}_2COOEt$), 1.82-1.62 (m, 4 H, 2 x $C\underline{H}_2$), 1.50-1.20 (m, 8 H, 4 x $C\underline{H}_2$), 1.27 (t, 3 H, *J* = 7.2 Hz, $C\underline{H}_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.62 ($\underline{C}OOEt$), 60.04 ($O\underline{C}H_2$), 34.23, 33.42, 30.33, 28.93 (2 x C), 28.23, 24.81, 14.21 ($\underline{C}H_3$), 7.04 ($I\underline{C}H_2$); MS (EI) calcd for $C_{11}H_{21}IO_2$ 312.0588, found 312.0590 (M).

Production and Isolation of 19, 20, 21, and 22. Fermentation of *S. cellulosae* was done in the usual fashion[60] except that in the final culture (700 mL) **6** was added in varying amounts (0.5, 1.0, 2.0, and 5.0 g/L) as a replacement for oleate esters (e.g., Span 85). After 7 days of fermentation the yellow orange cultures were extracted and the polyene fraction was purified through the LH-20 stage as before[60] to give 140 mg of pale yellow solid. This was dissolved in methanol (3 mL), water was added (2.7 mL), and the resulting precipitate was removed by centrifugation. HPLC separation (Waters C18 Radial Pak column, 60:40 methanol:water, flow 1.00 mL/min) of the supernatant afforded **22** (1.0 mg, retention time (R_t) = 8.37 min), **21** (3.3 mg, R_t = 10.43 min), **2** (2.1 mg, R_t = 16.1 min), **20** (1.0 mg, R_t = 16.1 min), and **19** (2.7 mg, R_t = 25.8 min). To separate **2** and **20**, which have identical retention times under these conditions, HPLC was repeated using 50:50 methanol:water (for **2**: R_t = 34.7 min; for **12**: R_t = 37.3 min).

Isochainin (19): mp 190 °C (dec); $[\alpha]_D$ -24.4 ° (*c* 0.16, MeOH); UV λ_{MAX} (THF/H_2O, 1/9) nm (ε) 307 (18,337), 321 (25,525), 338 (30,885), 357 (27,286); IR (MeOH cast) 3350 (br), 1723 (s), 1700 (s), 1046 (m), 848 (m) cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 6.51 (dd, 1 H, *J* = 14.6, 11.0 Hz), 6.25-6.43 (m, 6 H), 6.06 (d, 1 H, *J* = 11.0 Hz), 5.92 (dd, 1 H, *J* = 14.6, 6.0 Hz), 4.89 (m, 1 H), 4.15 (dd, 1 H, *J* = 10.4, 4.4 Hz), 3.91-4.04 (m, 5 H), 3.30 (m, 1 H), 3.81 (m, 1 H), 3.30 (m, 1 H), 2.31 (ddd, 1 H, *J* = 11.0, 7.0, 4.0 Hz), 1.77 (s, 3 H), 1.2-1.5 (m, 21 H), 0.90 (t, 3 H, *J* = 7.0 Hz); see Table 1 for ^{13}C NMR; positive ion FAB MS (glycerol) calcd for $C_{33}H_{54}O_{10}Na$ 633.36 , found 633.56 (MNa); calcd for $C_{33}H_{54}O_{10}$ 610.37, found 610.45 (M).

14-Hydroxyisochainin (20): UV λ_{MAX} (THF/H_2O, 1/9) nm (ε) 307 (27,180), 322 (35,789), 339 (45,313), 358 (41,403); IR (MeOH cast) 3260 (br), 1720 (s), 1705 (s), 1046 (m), 849 (m) cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 6.24-6.53 (m, 7 H), 6.06 (br d, 1 H, *J* = 11.4 Hz), 5.98 (dd, 1 H, *J* = 14.8, 5.2 Hz), 4.83 (m, 1 H), 3.69-4.04 (m, 8 H), 3.25 (m, 1 H), 2.34 (ddd, 1 H, *J* = 11.0, 7.0, 4.0 Hz), 1.78 (s, 3 H), 1.2-1.6 (m, 19 H), 0.90 (t, 3 H, *J* = 7.0 Hz); see Table 1 for ^{13}C NMR; positive ion FAB MS (glycerol) calcd for $C_{33}H_{54}O_{11}Na$ 649.36, found 649.55 (MNa); calcd for $C_{33}H_{54}O_{11}$ 626.36, found 626.50 (M).

1'-Hydroxyisochainin (21): UV λ_{MAX} (THF/H_2O, 1/9) nm (ε) 306 (12,197), 321 (16,360), 340 (17,045), 357 (15,682); IR (MeOH cast) 3343 (br), 1725 (s), 1705 (s), 845 (m) cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 6.25-6.53 (m, 7 H), 6.03 (br d, 1 H, *J* = 11.6 Hz), 5.98 (dd, 1 H, *J* = 14.6, 5.0 Hz), 4.88 (m, 1 H), 4.12 (dd, 1 H, *J* = 10.4, 5.6 Hz), 4.07 (br d, 1 H, *J* = 5.0 Hz), 3.82-4.21 (m, 6 H), 3.22 (dt, 1 H, *J* = 10.0, 2.5 Hz), 2.54 (dd, 1 H, *J* = 7.6, 7.2 Hz), 1.78 (s, 3 H), 1.27-1.54 (m, 19 H), 0.92 (t, 3 H, *J* = 7.0 Hz); see Table 1 for ^{13}C NMR; positive ion FAB MS (glycerol) calcd for $C_{33}H_{54}O_{11}Na$ 649.37, found 649.51 (MNa); calcd for $C_{33}H_{54}O_{11}$ 626.36, found 626.50 (M).

1',14-Dihydroxyisochainin (22): UV λ_{MAX} (THF/H_2O, 1/9) nm (ε) 306 (30,128), 322 (42,005), 340 (47,840), 358 (45,218); IR (MeOH cast) 3335 (br), 1723 (s), 1705 (s), 1049 (m), 845 (m) cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 6.22-6.51 (m, 7 H), 6.06 (br d, 1 H, *J* = 11.8 Hz), 6.01 (dd, 1 H, *J* = 14.4, 5.0 Hz), 4.79 (m, 1 H), 4.10 (br d, 1 H, *J* = 4.6 Hz), 3.90 (br d, 1 H, *J* = 9.0 Hz), 3.82-4.22 (m, 6 H), 3.71 (dd, 1 H, *J* = 9.0, 1.8 Hz), 3.27 (br d, 1 H, *J* = 11.0 Hz), 2.56 (dd, 1 H, *J* = 7.6, 7.0 Hz), 1.79 (s, 3 H), 1.11-1.70 (m, 17 H), 0.90 (t, 3 H, *J* = 7.0 Hz); see Table 1 for ^{13}C NMR; positive ion FAB MS (glycerol) calcd for 665.69 $C_{33}H_{54}O_{12}Na$ 665.35, found (MNa); calcd for $C_{33}H_{54}O_{12}$ 642.36, found 642.48 (M).

Preliminary Tests of Antifungal Activity of Compounds 19-22. The disk diffusion method of Boyer[204] was used to compare the antifungal activity of **19**, **20**, **21**, **22**, and amphotericin B (Sigma Chemical Co.). Five fungal strains, obtained from Professor Michael A. Pickard (University of Alberta Microbiology Department), were examined: *Aspergillus terreus* 327, *Cryptococcus ater* 164, *Mucor spp.*, *Tolypocladium niveum* UAMH2742, and *Torulopsis utilis var major* IMI33552. The surfaces of sterile agar plates (Difco potato dextrose agar, 39 g/L) were inoculated with suspensions of these organisms and paper disks containing concentrations of 40 μg/mL, 10 μg/mL, and 3 μg/mL of antibiotic were placed on the surface. Plates were allowed to prediffuse at 4 °C for 2 h before incubation at 30 °C. Diameters of inhibition zones were measured after 16 h, 24 h, and 32 h. All compounds showed antifungal activity at 3 μg/mL against all organisms with the following exceptions: *C. ater* was resistant in this assay to all compounds tested including amphotericin B; compounds **21** and **22** did not inhibit *T. utilis* at up to 40 μg/mL; *A. terreus* was only inhibited at 20 μg/mL (or more) by **19** and **20** and at 40 μg/mL by **21**, **22**, and amphotericin B.

Ethyl 9-Oxononanoate (24). The procedure of Crombie *et al.* was used.[86] Ozone (12-16 mg O_3/min) was bubbled through a solution of ethyl oleate **5** (7.00 g, 23.0 mmol) in EtOH (100 mL) at -60 °C for 2.5 h (until the solution turned a small amount of iodine in acetic acid from blue to colorless). Oxygen was passed through the solution for 10 min to remove the excess ozone before addition of dimethylsulfide (8.00 mL, 111 mmol). The reaction was stirred for 1 h at -10 °C and 12 h at room temperature. After removal of the solvent, the resulting residue was dissolved in hexanes (100 mL), washed with saturated $NaHCO_3$ solution (3 x 100 mL), H_2O (3 x 100 mL), dried (Na_2SO_4), and concentrated *in vacuo* to give a clear liquid (5.80 g), which was purified by flash column chromatography (silica, 10% EtOAc in hexanes) to give **24** (3.00 g, 67%) along with nonanal (**25**) (2.01 g, 63%). For **24**: IR ($CHCl_3$ cast) 2980 (m), 2933 (m), 2857 (m), 1735 (s) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 9.79 (t, 1 H, J = 1.7 Hz, $C\underline{H}O$), 4.12 (q, 2 H, J = 7.1 Hz, $OC\underline{H}_2$), 2.42 (m, 2 H, $C\underline{H}_2CHO$), 2.27 (t, 2 H, J = 7.0 Hz, $C\underline{H}_2COOEt$), 1.50 (m, 4 H), 1.30 (m, 6 H), 1.24 (t, 3 H, J = 7.1 Hz, $CH_2C\underline{H}_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.46 ($\underline{C}HO$), 173.52 ($\underline{C}OOEt$), 59.94 ($O\underline{C}H_2$), 43.62 ($OHC\underline{C}H_2$), 34.06, 29.00, 28.79, 28.69, 24.66, 21.78, 14.05 ($\underline{C}H_3$); MS (EI) calcd for $C_9H_{20}O_3$ 200.1365, found 200.1365 (M).

For (**25**): IR ($CHCl_3$ cast) 2956 (m), 2926 (m), 2872 (m), 1710 (s) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.75 (t, 1 H, *J* = 1.7 Hz, $C\underline{H}O$), 2.43 (dt, 2 H, *J* = 7.2, 1.7 Hz, $C\underline{H}_2CHO$), 1.63 (m, 2 H), 1.42-1.20 (m, 10 H), 0.88 (t, 3 H, *J* = 6.9 Hz, $C\underline{H}_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.54 ($\underline{C}HO$), 43.78 ($OHC\underline{C}H_2$), 31.70, 29.23, 29.07, 28.84, 22.32, 21.98, 14.10 ($\underline{C}H_3$); MS (EI) calcd for $C_9H_{17}O$-H 141.1279, found 141.1281 (M-H).

9-(*tert*-Butyldimethylsiloxy)nonyl Bromide (26). A modification of the procedure of Corey *et al.* was used.[92] A mixture of 9-bromononanol (**14**) (3.35 g, 15.0 mmol), *tert*-butyldimethylsilyl chloride (2.71 g, 18.0 mmol), and imidazole (2.55 g, 37.5 mmol) in DMF (25 mL) was stirred for 24 h at room temperature. Hexane (250 mL) was added to the mixture and this was washed with aqueous 5% $NaHCO_3$ (500 mL), H_2O (2 x 500 mL), dried (Na_2SO_4), and concentrated *in vacuo* to afford a yellow liquid (4.00 g). The residue was purified on an alumina column (20% ether in hexane) to give **26** (3.29 g, 65%). IR ($CHCl_3$ cast) 2953 (s), 2929 (s), 2856 (s) , 1101 (s) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.55 (t, 2 H, *J* = 6.7 Hz, $C\underline{H}_2OSi$), 3.48 (t, 2 H, *J* = 6.7 Hz, $BrC\underline{H}_2$), 1.72 (m, 2 H), 1.50-1.20 (m, 12 H), 0.88 (s, 9 H, $(C\underline{H}_3)_3C$), 0.06 (s, 6 H, $(C\underline{H}_3)_2Si$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 63.28 ($\underline{C}H_2OSi$), 45.10, 32.88, 32.68, 29.47, 29.35, 28.87, 26.91, 26.00 ($(\underline{C}H_3)_3C$), 25.79, 18.39 ($(CH_3)_3\underline{C}$), -5.25 and -5.63 ($(\underline{C}H_3)_2Si$); positive ion FAB MS (glycerol) calcd for $C_{15}H_{33}O^{79}BrSi$, found 336.97 (M); calcd for $C_{15}H_{33}O^{81}BrSi$, found 338.99 (M+2) .

9-(*tert*-Butyldimethylsiloxy)nonyl Iodide (27). The procedure of Crombie *et al.* was used.[86] A mixture of **26** (670 mg, 2.00 mmol) and NaI (360 mg, 2.40 mmol) in 2-butanone (10 mL) was heated under reflux for 20 h, then H_2O (20 mL) was added to the mixture. The aqueous phase was then extracted with CH_2Cl_2 (3 x 20 mL). The dried (Na_2SO_4) organic phases were concentrated and the residue was distilled

in vacuo to afford **27** (600 mg, 91%). Bp 170 °C (3.7 mm Hg); IR ($CHCl_3$ cast) 2954 (s), 2928 (s), 2856 (s) , 1255 (s), 1101 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.55 (t, 2 H, *J* = 6.5 Hz, $C\underline{H}_2OSi$), 3.16 (t, 2 H, *J* = 7.0 Hz, $IC\underline{H}_2$), 1.78 (m, 2 H), 1.60-1.20 (m, 12 H), 0.88 (s, 9 H, $(C\underline{H}_3)_3C$), 0.06 (s, 6 H, $(C\underline{H}_3)_2Si$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 63.32 ($\underline{C}H_2OSi$), 33.62, 32.90, 30.54, 29.43, 29.36, 28.53, 26.03 ($(\underline{C}H_3)_3C$), 25.80, 18.41 ($(CH_3)_3\underline{C}$), -5.23 ($(\underline{C}H_3)_2Si$); MS (EI) calcd for $C_{11}H_{24}OISi$ 327.0644, found 327.0643 ($M-C_4H_9$).

Ethyl 18-(*tert*-Butyldimethylsilyoxy)oleate (28). The method used for preparation of **6** was adopted. Thus, the Wittig reagent (0.680 mmol) derived from **27** reacted with the aldehyde (**24**) (130 mg, 0.670 mmol) to afford **28** (30.0 mg, 10%), after column chromatography (silica, 10% EtOAc in hexanes, R_f 0.85). IR ($CHCl_3$ cast) 2954 (s), 2928 (s), 2856 (s), 1255 (s), 1101 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.35 (m, 2 H, $C\underline{H}=C\underline{H}$), 4.13 (q, 2 H, *J* = 7.4 Hz, $OC\underline{H}_2$), 3.60 (t, 2 H, *J* = 6.7 Hz, $C\underline{H}_2OSi$), 2.28 (t, 2 H, *J* = 8.0 Hz, $C\underline{H}_2COOEt$), 2.01 (m, 4 H), 1.65-1.47 (m, 4 H), 1.30 (m, 18 H), 1.24 (t, 3 H, *J* = 7.4 Hz, $CH_2C\underline{H}_3$), 0.91 (s, 9 H, $(C\underline{H}_3)_3C$), 0.04 (s, 6 H, $(C\underline{H}_3)_2Si$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.89 ($\underline{C}OOEt$), 130.00, 129.81, 63.37 ($\underline{C}H_2OSi$), 60.16 ($O\underline{C}H_2$), 34.43, 32.94, 29.80, 29.73, 29.58, 29.46, 29.30, 29.21 (2 x C), 27.26, 27.22, 26.03 ($(\underline{C}H_3)_3C$), 25.85, 25.03, 18.41 ($(CH_3)_3\underline{C}$), 14.30 ($\underline{C}H_3$), -5.21 and 5.54 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) 441 (MH^+, 100).

NAC (*S*)-[2,3-$^{13}C_2$]-3-Hydroxybutyrate (34d). A procedure similar to that of Martin was used.[110] In a typical experiment, to a solution of labeled THP ether **49d** (2.36 g, crude) in dry MeOH (20 mL) was added CF_3COOH (3 drops) and the resulting solution was stirred for 17 h at 22 °C. After removal of the solvent *in vacuo*, a yellowish oil (1.82 g) was obtained. Purification of the residue by column chromatography (silica, 3.5 x 14 cm, EtOAc) afforded the labeled β-hydroxy thioester **34d**

(880 mg, R_f 0.15 in EtOAc, 32% yield). IR ($CHCl_3$ cast) 1653 (s), 1550 (m), 1290 (m), 1037 (m) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.22 (br s, 1 H, N$\underline{H}$), 4.45 and 4.06 (dm, 1 H, J = 145 Hz, ^{13}C$\underline{H}$(OH)), 3.43 (m, 2 H, C$\underline{H}_2$NH), 3.05 (dt, 2 H, J = 6.4, 4.2 Hz, SC$\underline{H}_2$), 2.88 and 2.57 (dm, 2 H, J = 129 Hz, ^{13}C$\underline{H}_2$), 2.84 (br s, 1 H, O$\underline{H}$), 2.00 (s, 3 H, COC$\underline{H}_3$), 1.24 (dt, 3 H, J = 6.2, 4.7 Hz, C$\underline{H}_3{}^{13}$CH(OH)); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.31(d, J = 46.0 Hz, $\underline{C}$OS), 170.48 (NH$\underline{C}$O), 64.45 (d, J = 36.0 Hz, enriched, $CH_3{}^{13}$$\underline{C}$H(OH)), 52.46 (d, J = 36.0 Hz, enriched, ^{13}$\underline{C}H_2$), 39.22 (CO$\underline{C}H_3$), 28.78 (S$\underline{C}H_2$), 23.16 ($\underline{C}H_2$NH), 22.70 (t, J = 19.6 Hz, $\underline{C}H_3{}^{13}$CH(OH)); MS (CI, NH_3) 208 (MH^+, 100), 225 ($MNH_4{}^+$, 96).

For unlabeled racemic material (**34a**): IR ($CHCl_3$ cast) 3280 (br), 1687 (s), 1657 (s), 1551 (m), 1290 (m), 1004 (m) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.12 (br s, 1 H, N$\underline{H}$), 4.26 (tq, 1 H, J = 6.3, 1.3 Hz, CH_3C$\underline{H}$(OH)), 3.45 (m, 2 H, C$\underline{H}_2$NH), 3.05 (dt, 2 H, J = 6.3, 3.6 Hz, SC$\underline{H}_2$), 2.75 (m, 2 H, C$\underline{H}_2$COO), 1.95 (br s, 3 H, COC$\underline{H}_3$), 1.35 (d, 3 H, J = 6.3 Hz, C$\underline{H}_3$CH(OH)); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.25($\underline{C}$OS), 170.55 (NH$\underline{C}$O), 64.98 ($CH_3$$\underline{C}$H(OH)), 52.46 ($\underline{C}H_2$COS), 39.18 (CO$\underline{C}H_3$), 28.74 (S$\underline{C}H_2$), 23.12 ($\underline{C}H_2$NH), 22.69 ($\underline{C}H_3$CH(OH)); MS (CI, NH_3) 206 (MH^+, 59), 223 ($MNH_4{}^+$, 100); Anal. Calcd for $C_{11}H_{20}O_4$: C, 46.81; H, 7.37; N, 6.82. Found: C, 46.72; H, 7.28; N, 6.51.

NAC (*S*)-[2,3-$^{13}C_2$,3-^{2}H]-3-Hydroxybutyrate (34e). The method for the conversion of **49d** to **34d** was used. Thus, **49e** (27.0 mg, 0.0994 mmol) gave **34e** (15.0 mg, 13% overall yield from **40f**), after preparative TLC (silica, EtOAc) purification. IR ($CHCl_3$ cast) 3300 (br), 3080 (m), 2968 (m), 2920 (m), 1682 (sh), 1659 (s), 1550 (m) cm^{-1} ; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.85 (br s, 1 H, N$\underline{H}$), 3.46 (m, 2 H, C$\underline{H}_2$NH), 3.05 (t, 2 H, J = 6.4 Hz, SC$\underline{H}_2$), 2.89 and 2.56 (dm, 2 H, J = 128 Hz, ^{13}C$\underline{H}_2$), 2.84 (br s, 1 H, O$\underline{H}$), 1.97 (s, 3 H, COC$\underline{H}_3$), 1.24 (br d, 3 H, J = 4.5 Hz, C$\underline{H}_3{}^{13}$CD(OH)); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.42 (t, J = 23.2 Hz, $\underline{C}$OS), 170.46 (NH$\underline{C}$O), 64.62 (dt,

J = 36.6, 22.3 Hz, enriched, $\mathrm{CH_3{}^{13}\underline{C}D(OH)}$), 52.31 (d, J = 36.5 Hz, enriched, $\mathrm{{}^{13}\underline{C}H_2}$), 39.30 ($\mathrm{CO\underline{C}H_3}$), 28.82 ($\mathrm{S\underline{C}H_2}$), 23.20 ($\mathrm{\underline{C}H_2NH}$), 22.56 (t, J = 19.7 Hz, $\mathrm{\underline{C}H_3{}^{13}CD(OH)}$); MS (CI, $\mathrm{NH_3}$) 209 ($\mathrm{MH^+}$, 89), 226 ($\mathrm{MNH_4{}^+}$, 67).

NAC *(S)*-[2,3-$^{13}\mathrm{C}_2$]-5-Hydroxyhex-2-enoate (35d). The method of Corey *et al.* was used.[121] The silyl ether **57d** (808 mg, 2.33 mmol) and AG 50W-X8 ion exchange resin (Bio-Rad, 1.7 meq/mL, 8.10 mL, 13.8 mmol) were added to a flask containing MeOH (60 mL). The mixture was heated at 40 °C for 30 min and filtered. The filtrate was concentrated to give a liquid residue (670 mg), which after purification by flash chromatography (silica, 2 x 6 cm, EtOAc) gave **35d** (425 mg, 78%, R_f 0.15). $[\alpha]_D$ +8.0° (c 0.48, $\mathrm{CHCl_3}$); IR ($\mathrm{CHCl_3}$ cast) 3280 (br), 1656 (s), 1579 (m), 1550 (m) cm^{-1}; ^{1}H NMR (360 MHz, $\mathrm{CDCl_3}$) δ 7.16 and 6.73 (ddt, 1 H, J = 136, 15.0, 7.7 Hz, $\mathrm{{}^{13}C\underline{H}{=}{}^{13}CHCOS}$), 6.45 and 6.00 (ddt, 1 H, J = 154, 15.0, 1.9 Hz, $\mathrm{{}^{13}CH{=}{}^{13}C\underline{H}COS}$), 5.91 (br s, 1 H, $\mathrm{N\underline{H}}$), 4.13 (m, 1 H, $\mathrm{C\underline{H}OH}$), 3.47 (dt, 2 H, J = 6.4, 5.5 Hz, $\mathrm{C\underline{H}_2NH}$), 3.11 (t, 2 H, J = 6.4 Hz, $\mathrm{SC\underline{H}_2}$), 2.38 (m, 2 H, $\mathrm{C\underline{H}_2{}^{13}CH}$), 1.97 (s, 3 H, $\mathrm{COC\underline{H}_3}$), 1.25 (d, 3 H, J = 6.3 Hz, $\mathrm{C\underline{H}_3CH(OH)CH_2}$); ^{13}C NMR (90 MHz, $\mathrm{CDCl_3}$) δ 190.04 (d, J = 62.0 Hz, $\mathrm{\underline{C}OS}$), 170.57 ($\mathrm{\underline{C}OCH_3}$), 142.53 (d, J = 69.6 Hz, enriched, $\mathrm{{}^{13}\underline{C}H{=}{}^{13}CHCOS}$), 130.25 (d, J = 69.6 Hz, enriched, $\mathrm{{}^{13}CH{=}{}^{13}\underline{C}HCOS}$), 66.40 ($\mathrm{\underline{C}H(OH)}$), 41.65 (d, J = 42.6 Hz, $\mathrm{\underline{C}H_2{}^{13}CH}$), 39.53 ($\mathrm{CO\underline{C}H_3}$), 28.19 ($\mathrm{S\underline{C}H_2}$), 23.27 (d, J = 3.7 Hz, $\mathrm{\underline{C}H_3CH(OH)CH_2}$), 23.04 ($\mathrm{\underline{C}H_2NH}$); MS (CI, $\mathrm{NH_3}$) 234 ($\mathrm{MH^+}$, 100), 251 ($\mathrm{MH_4{}^+}$, 83).

For unlabeled racemic material (**35a**): IR ($\mathrm{CHCl_3}$ cast) 3285 (br), 1660 (s), 1632 (m), 1550 (m) cm^{-1}; ^{1}H NMR (360 MHz, $\mathrm{CDCl_3}$) δ 6.94 (dt, 1H, J = 15.6, 7.6 Hz, $\mathrm{C\underline{H}{=}CHCOS}$), 6.21 (dt, 1H, J = 15.6, 1.4 Hz, $\mathrm{CH{=}C\underline{H}COS}$), 5.92 (br s, 1H, $\mathrm{N\underline{H}}$), 4.00 (m, 1H, $\mathrm{C\underline{H}OH}$), 3.47 (dt, 2H, J = 6.3, 5.6 Hz, $\mathrm{C\underline{H}_2NH}$), 3.10 (t, 2H, J = 6.3 Hz, $\mathrm{SC\underline{H}_2}$), 2.37 (m, 2H, $\mathrm{C\underline{H}_2CH{=}CH}$), 1.97 (s, 3H, $\mathrm{COC\underline{H}_3}$), 1.26 (d, 3H, J = 6.4 Hz, $\mathrm{C\underline{H}_3CH(OSi)CH_2}$); ^{13}C NMR (90 MHz, $\mathrm{CDCl_3}$) δ 190.12 ($\mathrm{\underline{C}OS}$), 170.51 ($\mathrm{\underline{C}OCH_3}$), 142.51 ($\mathrm{\underline{C}H{=}CHCOS}$), 130.40 ($\mathrm{CH{=}\underline{C}HCOS}$), 66.48 ($\mathrm{\underline{C}H(OH)}$), 41.70 ($\mathrm{\underline{C}H_2CH{=}CH}$), 39.57 ($CO\underline{C}H_3$), 28.22 ($S\underline{C}H_2$), 23.30 ($\underline{C}H_3CH(OSi)CH_2$), 23.06 ($\underline{C}H_2NH$); MS (CI, NH_3) 232 (MH^+, 100); Anal. Calcd for $C_{10}H_{17}NO_3S$: C, 51.92; H, 7.41; N, 6.06; S, 13.86. Found: C, 51.22; H, 7.43; N, 6.03; S, 13.92.

NAC *(S)*-[1,2-$^{13}C_2$,1-^{14}C]-5-Hydroxyhex-2-enoate (35e). The method for conversion of **57d** to **35d** was used. Thus, the silyl ether **57e** (149 mg, 0.430 mmol) afforded **35e** (74.0 mg, 74%). IR ($CHCl_3$ cast) 3285 (br), 1655 (s), 1638 (S), 1600 (m), 1552 (m) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.94 (dt, 1H, *J* = 15.6, 7.7 Hz, $C\underline{H}={}^{13}CH$), 6.64 and 5.83 (dm, 1H, *J* = 160 Hz, $CH={}^{13}C\underline{H}$), 5.92 (br s, 1H, $N\underline{H}$), 4.03 (m, 1H, $C\underline{H}(OH)$), 3.47 (m, 2H, $C\underline{H}_2NH$), 3.13 (m, 2H, $SC\underline{H}_2$), 2.38 (m, 2H, $C\underline{H}_2CH={}^{13}CH$), 1.97 (s, 3H, $COC\underline{H}_3$), 1.26 (d, 3H, *J* =6.4 Hz, $C\underline{H}_3CH(OSi)$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.07 (d, *J* = 61.4 Hz, enriched, $^{13}\underline{C}O$), 170.23 ($\underline{C}OCH_3$), 142.18 (d, *J* = 69.4 Hz, $\underline{C}H=13CH$), 130.54 (d, *J* = 61.4 Hz, $CH={}^{13}\underline{C}H$), 66.72 ($\underline{C}H(OH)$), 41.74 (d, *J* = 4.0 Hz, $\underline{C}H_2CH={}^{13}CH$), 39.74 ($CO\underline{C}H_3$), 28.38 ($S\underline{C}H_2$), 23.41 ($\underline{C}H_3CH(OH)$), 23.21 ($\underline{C}H_2NH$); MS (CI, NH_3) 234 (MH^+, 42), 251 (MNH_4^+, 100).

[1-^{13}C]Acetyl Chloride (37). The method used by Townsend *et al.* was followed.[107] Sodium [1-^{13}C]acetate (4.23 g, 50.9 mmol) (isotopic purity 99% ^{13}C) was carefully added to a flask containing phosphorus pentachloride (15.9 g, 76.4 mmol) to avoid a vigorous reaction. The mixture was heated to 80 °C under reflux for 10 min and then cooled to room temperature. Distillation under a stream of argon gave **37** (3.98 g, 98%). Bp 52 °C; ^{1}H NMR (360 MHz, $CDCl_3$) δ 2.66 (s, 3 H, $C\underline{H}_3$).

Ethyl [2-^{13}C]Acetate (38a). The procedure of Ropp *et al.* was used.[108a] Sodium [2-^{13}C]acetate (4.06 g, 48.9 mmol) (isotopic purity 99% ^{13}C) and triethyl phosphate (13.4 g, 73.3 mmol) were mixed and heated to reflux at 180 °C for 3 h.

Distillation under a stream of argon gave **38a** (3.90 g, 90%). Bp 76-78 ^{o}C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.10 (q, 2 H, *J* = 7.4 Hz, $OC\underline{H}_2$), 2.34 and 1.70 (d, 3 H, *J* = 128 Hz, $^{13}C\underline{H}_3$), 1.24 (t, 3 H, *J* = 7.4 Hz, $CH_2C\underline{H}_3$); ^{13}C NMR (90 MHz, $CDCl_3$) δ 170.96 (d, *J* = 60.0 Hz, $\underline{C}OO$), 60.27 ($\underline{C}H_2CH_3$), 20.90 (enriched, $^{13}\underline{C}H_3$), 14.10 ($CH_2\underline{C}H_3$).

Ethyl [1-^{13}C]Acetate (38b). The method used to prepare **38a** from sodium acetate was used. Thus, sodium [1-^{13}C]acetate (4.06 g, 48.9 mmol) (isotopic purity 99% ^{13}C) gave **38b** (4.06 g, 93%). Bp 76-78 ^{o}C.

Ethyl [1-^{13}C]Acetoacetate (39c). The procedure used to prepare **39d** was adopted. Thus, ethyl [1-^{13}C]acetate (4.06 g, 45.6 mmol) afforded ethyl [1-^{13}C] acetoacetate (**39c**) (3.73 g, 62%), after distillation. Bp 53-55 ^{o}C (7 mm Hg); IR (neat) 2977 (m), 2929 (m), 1729 (s), 1691 (s), 1255 (m) cm-1; ^{1}H NMR (360 MHz, $CDCl_3$) δ 4.20 (m, 2 H, $OC\underline{H}_2$), 3.45 (d, *J* = 2 H, 7.3 Hz, $C\underline{H}_2{}^{13}CO$), 2.27 (s, 3 H, $C\underline{H}_3CO$), 1.27 (t, 3 H, *J* = 7.0 Hz, $CH_2C\underline{H}_3$); ^{13}C NMR (90 MHz, $CDCl_3$) δ 200.65 ($CH_3\underline{C}O$), 167.14 (enriched, $^{13}\underline{C}OO$), 61.35 ($O\underline{C}H_2$), 50.07 (d, *J* = 58.6 Hz, $\underline{C}H_2{}^{13}COO$), 30.08 ($\underline{C}H_3CO$), 14.05 ($CH_2\underline{C}H_3$); MS (EI) calcd for $^{13}C_1C_5H_{10}O_3$ 131.0663, found 131.0663 (M).

Ethyl [2,3-$^{13}C_2$]Acetoacetate (39d). A procedure similar to that employed by Cane and Block was used.[109] LiHMDS was formed by the addition of *n*-BuLi (1.54 M, 115 mL, 177 mmol) to HMDS (38.0 mL, 29.2 g, 181 mmol) in dry THF (40 mL) at -78 ^{o}C. Ethyl [2-^{13}C]acetate **38a** (7.16 g, 80.4 mmol) was added to the LiHMDS solution at -78 ^{o}C and stirred for 15 min. [1-^{13}C]Acetyl chloride **37** (8.87, 112 mmol) was introduced dropwise over 10 min at -78 ^{o}C, and the mixture was stirred for 1h. The mixture was then treated with 2N HCl (200 mL) at -78 ^{o}C. After warming to 20 ^{o}C, the reaction solution was extracted with ether (3 x 100 mL). The organic phases were combined, washed with 1 N HCl (100 mL), saturated solution of $NaHCO_3$ (100 mL), brine (100 mL), dried (Na_2SO_4), and concentrated *in vacuo*. The resulting yellow residue (14.0 g) was distilled to afford **39d** (6.70 g, 63%). Bp 78-80 °C (15 mm Hg); IR ($CHCl_3$ cast) 2960 (m), 1740 (s), 1679 (m), 1138 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.21 (q, 2 H, J = 7.1 Hz, $O\underline{C}\underline{H}_2$), 3.62 and 3.29 (dd, 2 H, J = 130, 6.3 Hz, $^{13}C\underline{H}_2$), 2.28 (dd, 3 H, J = 6.1, 1.4 Hz, $C\underline{H}_3{}^{13}CO$), 1.29 (t, 3H, J = 7.0 Hz, $CH_2C\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 200.63 (d, J = 38.0 Hz, enriched, $^{13}\underline{C}O$), 167.02 ($\underline{C}OO$), 61.37 ($O\underline{C}H_2$), 50.13 (d, J = 38.0 Hz, enriched, $^{13}\underline{C}H_2$), 26.93 ($\underline{C}H_3{}^{13}CO$), 14.06 ($CH_2\underline{C}H_3$); MS (EI) calcd for $^{12}C_4{}^{13}C_2H_{10}O_3$ 132.0697, found 132.0698 (M).

Ethyl (*S*)-[1-^{13}C]-3-Hydroxybutyrate (40c). The method used to prepare **40d** from **39d** was used. Thus, **39c** (2.60 g, 19.0 mmol) gave **40d** (1.33 g, 50%). Bp 61 °C (3.0 mm Hg); $[\alpha]_D$ +34.0° (*c* 1.49, $CHCl_3$); IR ($CHCl_3$ cast) 3440 (br), 2975 (m), 2873 (m), 1691 (s) cm-1; ^1H NMR (360 MHz, $CDCl_3$) δ 4.3-4.1 (m, 3 H, $C\underline{H}(OH)$ and $OC\underline{H}_2$), 2.44 (m, 2 H, $C\underline{H}_2{}^{13}CO$), 1.24 (t, 3 H, J = 7.0 Hz, $CH_2C\underline{H}_3$), 1.17 (d, 3 H, J = 6.0 Hz, $C\underline{H}_3CH(OH)$); ^{13}C NMR (90 MHz, $CDCl_3$) δ 172.75 (enriched, $^{13}\underline{C}OO$), 64.15 ($\underline{C}H(OH)$), 60.51 (d, J = 2.4 Hz, $O\underline{C}H_2$), 42.60 (d, J = 57.4 Hz, $\underline{C}H_2{}^{13}COO$), 22.37 (d, J = 4.9 Hz, $\underline{C}H_3CH(OH)$), 14.05 ($CH_2\underline{C}H_3$); MS (EI) calcd for $^{13}C_1C_5H_{12}O_3$ 133.0820, found 133.0801 (M).

Ethyl (*S*)-[2,3-^{13}C$_2$]-3-Hydroxybutyrate (40d). A modification of the procedure of K. Mori was employed.[112] In a typical experiment, the labeled ethyl [2-^{13}C]acetoacetate (1.60 g, 12.1 mmol) in 98% EtOH (10 mL) was added to a vigorously stirred solution of glucose (22.0 g) and baker's yeast (20.0 g) in 0.1M potassium phosphate buffer (320 mL, pH 7) at 30 °C. The solution was stirred at 30 °C for ca. 4 h until there was no starting material left as determined by TLC. Celite 545 (20 g) and ether (100 mL) were added to the solution, and the mixture was filtered through a pad of Celite 545. The filtrate was then extracted with ether (6 x 200 mL). The combined organic phases were washed with brine (200 mL), dried (Na_2SO_4), and concentrated. The resulting liquid residue (ca. 100 mL) was distilled under reduced pressure to yield **40d** (1.55 g, 95%). Bp 70-72 ^oC (10 mm Hg); $[\alpha]_D$ +36.2^o (*c* 1.00, $CHCl_3$); IR ($CHCl_3$ cast) 3440 (br), 2978 (m), 2938 (m), 1735 (s) cm-1; ^1H NMR (400 MHz, $CDCl_3$) δ 4.38 and 4.02 (dm, 1 H, *J* = 155 Hz, $^{13}\mathrm{C\underline{H}(OH)}$), 4.18 (q, 2 H, *J* = 7.2 Hz, $\mathrm{OC\underline{H}_2}$), 2.66 and 2.34 (dddd, 1 H, *J* = 128, 16.6, 3.3, 2.6 Hz, $^{13}\mathrm{C\underline{H}H}$), 2.57 and 2.25 (dddd, 1 H, *J* = 128, 16.6, 8.7, 5.6 Hz, $^{13}\mathrm{CH\underline{H}}$), 2.50 (br s, 1 H, $\mathrm{O\underline{H}}$), 1.28 (t, 3 H, *J* = 7.1 Hz, $\mathrm{CH_2C\underline{H}_3}$), 1.23 (dt, 3 H, *J* = 6.3, 4.7 Hz, $\mathrm{C\underline{H}_3{}^{13}CH(OH)}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.97 (t, *J* = 28.5 Hz, $\mathrm{\underline{C}OO}$), 64.23 (d, *J* = 38.2 Hz, enriched, $^{13}\mathrm{\underline{C}H(OH)}$), 60.66 ($\mathrm{O\underline{C}H_2}$), 42.49 (d, *J* = 38.2 Hz, enriched, $^{13}\mathrm{\underline{C}H_2}$), 22.36 (t, *J* = 19.6 Hz, $\mathrm{\underline{C}H_3{}^{13}CH(OH)}$), 14.15 ($\mathrm{CH_2\underline{C}H_3}$); MS (CI, NH_3) 135 (MH^+, 41), 152 (MNH_4^+, 100).

For unlabeled material **40b** (ethyl (*S*)-3-hydroxybutyrate): $[\alpha]_D$ +36.4^o (*c* 1.06, $CHCl_3$); IR ($CHCl_3$ cast) 3445 (br), 2978 (m), 2929 (m), 1735 (s) cm-1; ^1H NMR (400 MHz, $CDCl_3$) δ 4.20 (m, 1 H, $\mathrm{C\underline{H}(OH)}$), 4.19 (q, 2 H, *J* = 7.1 Hz, $\mathrm{OC\underline{H}_2}$), 2.49 (dd, 1 H, *J* = 16.5, 3.6 Hz, $\mathrm{C\underline{H}HCO}$), 2.41 (dd, 1 H, *J* = 16.5, 8.7 Hz, $\mathrm{CH\underline{H}CO}$), 1.28 (t, 3 H, *J* = 7.1 Hz, $\mathrm{CH_2C\underline{H}_3}$), 1.23 (d, 3 H, *J* = 6.3 Hz, $\mathrm{C\underline{H}_3CH(OH)}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.59 ($\mathrm{\underline{C}OO}$), 64.07 ($\mathrm{\underline{C}H(OH)}$), 60.41 ($\mathrm{O\underline{C}H_2}$), 42.82 ($\mathrm{\underline{C}H_2CO}$), 22.32 ($\mathrm{\underline{C}H_3CH(OH)}$), 13.95 ($\mathrm{CH_2\underline{C}H_3}$); MS (EI) calcd for $C_6H_{12}O_3$ 133.0865, found 133.0863 (M).

Ethyl [2,3-$^{13}C_2$, 3-^2H]-3-Hydroxybutyrate (40e). A modification of the method of De Koning *et al.* was adopted.[122a] Thus, the β-ketoester **39d** (900 mg, 6.81 mmol) was dissolved in dry EtOH (5 mL), and to this solution $NaBD_4$ (107 mg, 10.2 mmol) (isotopic purity 99% $^2H_4$) in EtOH (3 mL) was added at 0 ^oC. The mixture was stirred for 1.5 h at 0 ^oC and 3.5 h at room temperature. Aqueous 0.5N HCl (50 mL) was added to the reaction mixture. The aqueous phase was extracted with ether (5 x 50 mL), the combined organic phases were dried over Na_2SO_4 and concentrated to afford a yellow liquid (5.3 g), which upon distillation gave **40e** (870 mg, 97%). IR ($CHCl_3$ cast) 3440 (br), 2980 (m), 2932 (m), 2918 (m), 1734 (s) cm-1; ^1H NMR (400 MHz, $CDCl_3$) δ 4.17 (q, 2 H, *J* = 7.2 Hz, $OC\underline{H}_2$), 2.65 and 2.34 (ddd, 1 H, *J* = 130, 16.5, 2.5 Hz, $^{13}C\underline{H}H$), 2.57 and 2.26 (dddt, 1 H, *J* = 130, 16.5, 5.5, 1.1 Hz, $^{13}CH\underline{H}$), 2.20 (br s, 1 H, $O\underline{H}$), 1.28 (t, 3 H, *J* = 7.1 Hz, $CH_2C\underline{H}_3$), 1.22 (br t, 3 H, *J* = 4.7 Hz, $C\underline{H}_3{}^{13}CD(OH)$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.96 (t, *J* = 28.7 Hz, $\underline{C}OO$), 63.81 (dt, *J* = 37.9, 22.1 Hz, enriched, $^{13}\underline{C}D(OH)$), 60.66 ($O\underline{C}H_2$), 42.30 (d, *J* = 36.9 Hz, enriched, $^{13}\underline{C}H_2$), 22.24 (t, *J* = 19.8 Hz, $\underline{C}H_3{}^{13}CD(OH)$), 14.14 ($CH_2\underline{C}H_3$); MS (CI, NH_3) 136 (MH^+, 100), 153 (MNH_4^+, 82).

Ethyl (*S*)-[2,3-$^{13}C_2$, 3-^2H]-3-Hydroxybutyrate (40f). A modification of the procedure of Klibanov and coworkers was used.[193] A mixture of racemic ethyl 3-hydroxybutryate **40e** (670 mg, 4.96 mmol), trichloroethyl butyrate **131**(1.31 g, 5.97 mmol), and porcine pancreatic lipase (5.10 g, predried 3 days at high vacuum before use) in dry ether (20 mL) was stirred at room temperature for 20 h (until 50% of the **40e** was gone by GC). The mixture was filtered and the filtrate was concentrated to give a liquid (1.95 g), which was chromatographed (silica, 3 x 20 cm, 50% ether in pentane) to afford **40f** (104 mg, 16%, R_f 0.66) (contaminated with 10-20% trichloroethanol), and pure **133e** (340 mg, 67%, Rf 0.74). For **133e**: $[\alpha]_D$ -0.92 (*c* 1.19, $CHCl_3$); IR ($CHCl_3$ cast) 2958 (m), 2929 (m), 1739 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.15 (t, 2 H, *J* = 7.1 Hz, $OC\underline{H}_2CH_3$), 2.78 and 2.46 (ddd, 1 H, *J* = 130, 15.5, 6.0 Hz, $^{13}C\underline{H}H$), 2.65 and 2.32 (ddd, 1 H, *J* = 128, 15.5, 3.5 Hz, $^{13}CH\underline{H}$), 2.25 (t, 2 H, *J* = 7.5 Hz, $OOCC\underline{H}_2$), 1.62 (m, 2 H, $OOCCH_2C\underline{H}_2$), 1.29 (br t, 3 H, *J* = 4.5 Hz, $C\underline{H}_3{}^{13}CD(O)$), 1.25 (t, 3 H, *J* = 7.1 Hz, $OCH_2C\underline{H}_3$), 0.92 (t, 3 H, *J* = 7.5 Hz, $CH_2CH_2C\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.72 ($OO\underline{C}CH_2$), 170.17 (t, *J* = 29.2 Hz, $^{13}CH_2\underline{C}O$), 66.65 (dt, *J* = 39.7, 23.0 Hz, enriched, $CH_3{}^{13}\underline{C}D(OH)$), 60.47 ($O\underline{C}H_2$), 40.75 (d, *J* = 39.6 Hz, enriched, $^{13}\underline{C}H_2$), 36.26, 19.70, 18.34, 14.06, 13.48; MS (CI, NH_3) 206 (MH^+, 53), 223 (MNH_4^+, 100).

For unlabeled compound: racemic ethyl 3-hydroxybutyrate (**40a**) (660mg, 4.99 mmol) was used and ethyl (*S*)-3-hydroxybutyrate (**40b**) (210 mg, 32%) was obtained, which showed identical MS, IR, ^{1}H and ^{13}C NMR spectra with racemic **40a**. $[\alpha]_D$ +41.9° (*c* 1.46, CHCl$_3$).

Ethyl (*S*)-[1-^{13}C]-3-(*tert*-Butyldimethylsiloxy)butyrate (41c). The procedure used by Seebach *et al.* was followed.[111] To a stirred solution of hydroxy ester **40c** (1.17 g, 8.79 mmol) in dry DMF (20 mL) was sequentially added *tert*-butyldimethylsilyl chloride (1.60 g, 10.6 mmol) and imidazole (900 mg, 13.2 mmol). The solution was stirred for 2 days at room temperature and poured into hexanes (100 mL). This was washed with H$_2$O (3 x 100 mL), dried (Na$_2$SO$_4$), and concentrated *in vacuo* to give pure silyl ether **41c** (2.15 g, 99%). $[\alpha]_D$ +22.3° (*c* 0.72, CHCl$_3$); IR (CHCl$_3$ cast) 2997 (w), 2930 (m), 2890 (m), 2858 (m), 1698 (s), 1255 (m) cm^{-1}; ^{1}H NMR (360 MHz, CDCl$_3$) δ 4.25 (m, 1 H, C$\underline{\mathrm{H}}$(OSi)), 4.10 (m, 2 H, OC$\underline{\mathrm{H}}_2$), 2.6-2.3 (m, 2 H, C$\underline{\mathrm{H}}_2{}^{13}$CO), 1.23 (t, 3 H, *J* = 6.8 Hz, CH$_2$C$\underline{\mathrm{H}}_3$), 1.17 (d, 3 H, *J* = 5.8 Hz, C$\underline{\mathrm{H}}_3$CH(OSi)), 0.88 (s, 9 H, (C$\underline{\mathrm{H}}_3$)$_3$C), 0.08 (s, 6 H, (C$\underline{\mathrm{H}}_3$)$_2$Si); ^{13}C NMR (90 MHz, CDCl$_3$) δ 171.66 (enriched, $^{13}\underline{\mathrm{C}}$OO), 65.84 ($\underline{\mathrm{C}}$H(OSi)), 60.20 (t, *J* = 2.4 Hz, O$\underline{\mathrm{C}}$H$_2$), 44.95 (d, *J* = 57.4 Hz, $\underline{\mathrm{C}}$H$_2{}^{13}$COO), 25.71 (($\underline{\mathrm{C}}$H$_3$)$_3$C), 23.89 (d, *J* = 3.7 Hz, $\underline{\mathrm{C}}$H$_3$CH(OSi)), 17.93 ((CH$_3$)$_3\underline{\mathrm{C}}$), 14.16 (CH$_2\underline{\mathrm{C}}$H$_3$), -4.53 and -5.05 (($\underline{\mathrm{C}}$H$_3$)$_2$Si); MS (CI, NH$_3$) 248 (M$^+$, 100).

Ethyl (*S*)-[2,3-^{13}C$_2$]-3-(*tert*-Butyldimethylsiloxy)butyrate (41d). The method for the conversion of **40c** to **41c** was used. Thus, the hydroxy compound **40d** (1.54 g, 11.5 mmol) gave **41d** (2.83 g, 99%). IR (CH$_2$Cl$_2$ cast) 2957 (m), 2930 (m), 2858 (m), 1740 (s), 1255 (m) cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 4.46 and 4.11 (dm, 1 H, *J* = 140 Hz, ^{13}C$\underline{\mathrm{H}}$(OSi)), 4.12 (dq, 2 H, *J* = 7.1, 4.0 Hz, OC$\underline{\mathrm{H}}_2$), 2.63 and 2.31 (dm, 1H, *J* = 130 Hz, ^{13}C$\underline{\mathrm{H}}$H), 2.52 and 2.21 (dm, 1 H, *J* = 127 Hz, ^{13}CH$\underline{\mathrm{H}}$), 1.27 (t, 3 H, *J*

= 7.1 Hz, $CH_2C\underline{H}_3$), 1.20 (dt, 3 H, *J* = 6.0, 4.4 Hz, $C\underline{H}_3{}^{13}CH(OSi)$), 0.89 (s, 9 H, $(C\underline{H}_3)_3C$), 0.08 (s, 6 H, $(C\underline{H}_3)_2Si$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.68 (t, *J* = 28.5 Hz, $\underline{C}OO$), 65.84 (d, *J* = 38.8 Hz, enriched, $^{13}\underline{C}H(OSi)$), 60.23 ($O\underline{C}H_2$), 44.95 (d, *J* = 38.9 Hz, $^{13}\underline{C}H_2$), 25.70 ($(\underline{C}H_3)_3C$), 23.70 (t, *J* = 19.5 Hz, $\underline{C}H_3{}^{13}CH(OSi)$), 17.92 ($(CH_3)_3\underline{C}$), 14.16 ($CH_2\underline{C}H_3$), -4.54 and -5.07 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) 249 (MH^+, 48).

***N*, *S*-Diacetyl-β-mercaptoethylamine (44).** The procedure of Gerstein *et al.* was used.[113a] To a solution of 2-mercaptoethylamine hydrochlororide (56.8 g, 500 mmol) in H_2O (150 mL) at -5 °C was added acetic anhydride (153 g, 150 mmol) and aqueous 8M KOH simultaneously over 110 min in such a way that the pH was maintained at 8. The mixture was stirred for 1 h at room temperature and extracted with ether (3 x 200 mL). The combined organic phases were dried over Na_2SO_4 and concentrated to afford a colorless liquid (78.4 g), which was distilled at reduced pressure to give pure **44** (67.7 g, 84%). Bp 138-141 °C (0.5 mm Hg); ^1H NMR (360 MHz, $CDCl_3$) δ 6.33 (br s, 1 H, $N\underline{H}$), 3.37 (m, 2 H, $C\underline{H}_2NH$), 2.99 (t, 2 H, *J* = 6.4 Hz, $SC\underline{H}_2$), 2.30 (s, 3 H, $C\underline{H}_3COS$), 1.95 (s, 3 H, $NHCOC\underline{H}_3$); ^{13}C NMR (90 MHz, $CDCl_3$) δ 196.01 ($S\underline{C}O$), 170.32 ($N\underline{C}O$), 39.36 ($NCO\underline{C}H_3$), 30.46 ($\underline{C}H_3COS$), 28.64 ($S\underline{C}H_2$), 22.98 ($\underline{C}H_2NH$).

***N*-Acetylcysteamine (45).** A modification of the method of Schwab *et al.* was followed.[113b] To a 0 °C solution of **44** (8.00g, 49.6 mmol) in H_2O (150 mL) was added solid KOH (9.00 g, 160 mmol) over 40 min. The mixture was stirred under argon for 2 h at room temperature, then neutralized to pH 7 with 2 N HCl and saturated with NaCl. The mixture was extracted with CH_2Cl_2 (5 x 50 mL). The combined organic phases were dried (Na_2SO_4) and concentrated to give essentially pure **45** (5.49 g, 93%). IR ($CHCl_3$ cast)

3288 (br), 1652 (s), 1549 (s), 1373 (m), 1280 (m); ^{1}H NMR (360 MHz, $CDCl_3$) δ 6.05 (br s, 1 H, N$\underline{H}$), 3.43 (dt, 2 H, *J* = 6.4, 5.9 Hz, $C\underline{H}_2NH$), 2.68 (ddt, 2 H, *J* = 8.3, 6.4, 1.9 Hz, $SC\underline{H}_2$), 2.02 (s, 3 H, $COC\underline{H}_3$), 1.36 (t, 1 H, *J* = 8.3 Hz, S$\underline{H}$); ^{13}C NMR (90 MHz, $CDCl_3$) δ 170.2 ($\underline{C}O$), 42.03 ($CO\underline{C}H_3$), 23.50, 22.28; MS (EI) calcd for C_4H_9NOS 119.0405, found 119.0402 (M).

Ethyl *(S)*-[2,3-$^{13}C_2$]-3-[(2-Tetrahydropyranyl)oxy]butyrate (46d). A procedure similar to the literature method was used.[118] To a stirred solution of hydroxy ester **40d** (1.80 g, 13.4 mmol) in dry CH_2Cl_2 (20 mL) at 0 °C was added 3,4-dihydro-2*H*-pyran (5.64 g, 67.1 mmol) over 5 min, followed by trifluoroacetic acid (2 drops). The solution was stirred overnight at room temperature. The volatile solvent was removed *in vacuo* to give essentially pure **46d** in quantitative yield. For unlabeled racemic material (**46a**): IR ($CHCl_3$ cast) 2925 (m), 1737 (s), 1032 (m), 1022 (m) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.75 and 4.69 (2 x d, 1 H, *J* = 4.7, 3.3 Hz, $OC\underline{H}O$), 4.25 (m, 1 H, $CH_3C\underline{H}(OCHO)$), 3.95-3.81 (m, 1 H, $CH_2C\underline{H}HO$), 3.48 (m, 3 H, $CH_2CH\underline{H}O$ + $OC\underline{H}_2CH_3$), 2.67 and 2.56 (dd, 1 H, *J* = 15.1, 7.4 Hz, $C\underline{H}HCOO$), 2.42 and 2.39 (dd, 1 H, *J* = 15.1, 6.0 Hz, $CH\underline{H}COO$), 1.9-1.47 (m, 6 H, 3 x $C\underline{H}_2$), 1.31-1.18 (2 x t + 2 x d, 6 H, *J* = 7.2, 6.3 Hz, $OCH_2C\underline{H}_3$ + $C\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.08 and 171.04 ($\underline{C}OO$), 98.89 and 95.54 ($O\underline{C}HO$), 70.32 and 68.20 ($CH_3\underline{C}H(OCH)$), 62.34 and 61.68 ($O\underline{C}H_2CH_2$), 59.91 ($O\underline{C}H_2CH_3$), 42.45 and 41.47 ($\underline{C}H_2COO$), 30.69 and 30.62, 25.15, 21.56 and 19.51, 19.07 and 19.00, 13.89 ($CH_2\underline{C}H_3$); MS (CI, NH_3) 217 (MH^+, 15), 234 (MNH_4^+, 47); Anal. Calcd for $C_{11}H_{20}O_4$: C, 61.09; H, 9.32. Found: C, 60.65; H, 9.01.

Ethyl (S)-[2,3-$^{13}C_2$, 3-^{2}H]-3-[(2-Tetrahydropyranyl)oxy]butyrate (**46e**). The method for the conversion of **40d** to **46d** was used. Thus, **40f** (100 mg, 0.651 mmol) afforded **46e** (170 mg, 99%), which was used without purification.

THP Ether of Sodium *(S)*-[2,3-$^{13}C_2$]-3-Hydroxybutyrate (47d). A procedure similar to that of Martin was used.[110] To an ice-cooled solution of THP ether **46d** (13.4 mmol, based on 100% conversion) in MeOH (25 mL) was added 2 N NaOH (10.1 mL, 20.2 mmol) over 10 min. The cooling bath was removed, and the solution stirred at 22 °C for 14 h. The MeOH was removed *in vacuo*, the residue was redissolved in H_2O (50 mL), and the aqueous phase was extracted with hexanes (2 x 50 mL). Removal of the H_2O *in vacuo* from the aqueous phase afforded **47d**, which was carried on to the next reaction without further purification.

Sodium (S)-[2,3-$^{13}C_2$,3-^{2}H]-3-[(2-Tetrahydropyranyl)oxy]butyrate (**47e**). The method for the conversion of **46d** to **47d** was employed. Thus, **46e** (100 mg, 0.651 mmol) gave **47e** (170 mg, 99%), which was used without purification.

Methoxycarbonyl *(S)*-[2,3-$^{13}C_2$]-3-[(2-Tetrahydropyranyl)oxy] butyrate (48d). A procedure similar to that of Martin was used.[110] To a suspension of the sodium salt **47d** (13.4 mmol, based on 100% conversion) in THF (50 mL) was added methyl chloroformate (2.30 mL, 29.7 mmol) and a catalytic amount of triethylamine (3 drops). A copious precipitate formed after the addition of the methyl chloroformate. The reaction was stirred for 20 h at room temperature, and then filtered through a pad of Celite 545. The filtrate was concentrated *in vacuo* to afford the mixed anhydride **48d**, which was immediately used for the next reaction.

Methoxycarbonyl (S)-[2,3-$^{13}C_2$,3-^{2}H]-3-[(2-Tetrahydropyranyl)oxy] butyrate (**48e**). The method for the conversion of **47d** to **48d** was used. Thus, **47e** (ca. 0.651 mmol, based on 100% conversion) gave **48e** (170 mg, 99%), which was used without purification.

NAC thioester of *(S)*-[2,3-$^{13}C_2$]-3-[(2-Tetrahydropyranyl)oxy] butyrate (49d). A procedure similar to that of Martin was used.[110] To a cold (0 °C) solution of the mixed anhydride **47d** (13.4 mmol) in dry THF (40 mL) was added *N*-acetylcysteamine (4.79 g, 40.2 mmol) and triethylamine (3.74 mL, 2.72 g, 26.9 mmol) simultaneously over 10 min. The mixture was warmed to room temperature, stirred overnight and the THF was removed *in vacuo*. The resulting residue was dissolved in EtOAc (100 mL) and washed with cold aqueous 1 N KOH (50 mL). Concentration of the dried (Na_2SO_4) organic phase afforded the crude product **49d** (2.36 g). For unlabeled racemic material (**49a**): IR ($CHCl_3$ cast) 3284 (br), 2940 (m), 1688 (s), 1656 (s), 1549 (m), 1120 (m), 1021 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.12 and 5.95 (2 x br s, 1 H, $N\underline{H}$), 4.70 and 4.65 (m, 1 H, $OC\underline{H}O$), 4.25 (m, 1 H, $CH_3C\underline{H}(OCHO)$), 3.92-3.77 (m, 1 H, $CH_2CH\underline{H}O$), 3.55-3.46 (m, 3 H, $CH_2C\underline{H}HO + C\underline{H}_2NH$), 3.05 (m, 2 H, $SC\underline{H}_2$), 2.9-2.6 (m, 2 H, $C\underline{H}_2COO$), 1.95 (br s, 3 H, $COC\underline{H}_3$), 1.9-1.5 (m, 6 H, 3 x $C\underline{H}_2$), 1.3-1.2 (2 x d, 3 H, J = 6.3 Hz, $C\underline{H}_3CH(OCHO)$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.80 and 197.61 ($\underline{C}OS$), 170.28 and 170.20 ($NH\underline{C}O$), 98.94 and 96.52 ($O\underline{C}HO$), 70.84 and 69.08 ($CH_3\underline{C}H(OCH)$), 62.70 and 62.60 ($O\underline{C}H_2CH_2$), 51.68 and 51.00, 39.60 and 39.49 ($\underline{C}H_2COS$), 31.05 and 30.84, 28.62 and 28.55, 25.36 and 25.33, 23.16, 21.80, 19.69 and 19.56; MS (Cl, NH_3) 290 (MH^+, 7), 307 (MNH_4^+, 30); Anal. Calcd for $C_{11}H_{20}O_4$: C, 53.96; H, 8.01; N, 4.84; S, 11.08. Found: C, 53.33; H, 8.17; N, 4.82; S, 11.11.

NAC (S)-[2,3-$^{13}C_2$,3-^2H]-3-[(2-Tetrahydropyranyl)oxy] butyrate (**49e**). The method for the conversion of **48d** to **49d** was used. Thus, **48e** (ca. 0.651 mmol, based on 100% conversion) gave **49e** (170 mg, 99%), which was used without purification.

(*S*)-3-Hydroxylbutyl Benzoate (50b). The method used by Seebach and coworkers was employed.[111] To a solution of (3*S*)-1,3-butanediol (7.21 g, 80.0 mmol) and dry pyridine (9.60 mL, 119 mmol) in CH_2Cl_2 (70 mL) was added benzoyl chloride (11.3 g, 80.0 mmol) in CH_2Cl_2 (25 mL) over 20 min at -45 °C. The reaction mixture was stirred for 2 h between -35 °C to -20 °C and then overnight at room temperature. The mixture was then poured into ice-cold 1 N HCl (150 mL), and the aqueous phase was extracted with CH_2Cl_2 (3 x 50 mL). The combined organic phases were dried (Na_2SO_4) and concentrated to afford a colorless liquid (18.9 g). Distillation *in vacuo* yielded **50b** (12.7 g, 82%). Bp 110-112 °C (0.5 mm Hg); $[\alpha]_D$ +30.0° (*c* 1.35, $CHCl_3$); IR (CH_2Cl_2 cast) 3335 (br), 2970 (m), 1719 (s), 1603 (w), 1586 (m), 1278 (s) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 8.06 (m, 2 H, 2-Ar$\underline{H}$), 7.6-7.4 (m, 3 H, 3-Ar$\underline{H}$), 4.62 and 4.40 (2 x m, 2 H, $C\underline{H}_2O$), 4.00 (m, 1 H, $C\underline{H}(OH)$), 2.36 (br s, 1 H, $O\underline{H}$), 2.0-1.8 (m, 2 H, $C\underline{H}_2CH_2O$), 1.28 (d, 3 H, *J* = 6.0 Hz, $C\underline{H}_3CH(OH)$); ^{13}C NMR (90 MHz, $CDCl_3$) δ 166.78 ($\underline{C}OO$), 132.83 (Ar$\underline{C}$), 129.96 (Ar$\underline{C}$), 128.20 (Ar$\underline{C}$), 128.26 (Ar$\underline{C}$), 64.55 ($\underline{C}H(OH)$), 62.06 ($\underline{C}H_2O$), 37.94 ($\underline{C}H_2CH_2O$), 23.34 ($\underline{C}H_3CH(OH)$); MS (CI, NH_3) 195 (MH^+, 100); Anal. Calcd for $C_{11}H_{14}O_3$: C, 68.02; H, 7.26. Found: C, 67.67; H, 7.04.

***(S)*-3-(*tert*-Butyldimethylsiloxy)butyl Benzoate (51b).** The procedure of Seebach and coworkers was followed.[111] The alcohol **50b** (11.7 g, 60.2 mmol), imidazole (6.17 g, 90.6 mmol), and *tert*-butyldimethylsilyl chloride (10.9 g, 72.3 mmol) were dissolved in dry DMF (120 mL). The mixture was stirred for 2 days at room temperature, then diluted with hexanes (500 mL) and washed with H_2O (3 x 150 mL). The organic phase was dried over $MgSO_4$ and concentrated *in vacuo* to give **51b** as a colorless liquid in quantitative yield. $[\alpha]_D$ +29.1° (*c* 1.39, $CHCl_3$); IR (CH_2Cl_2 cast) 2958 (m), 2925 (m), 2855 (m), 1722 (s), 1603 (w), 1276 (s) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.05 (m, 2 H, 2-Ar$\underline{H}$), 7.6-7.36 (m, 3 H, 3-Ar$\underline{H}$), 4.38 (m, 2 H, $C\underline{H}_2O$), 4.05 (m, 1 H, $C\underline{H}(OSi)$), 1.95-1.78 (m, 2 H, $C\underline{H}_2CH_2O$), 1.20 (d, 3 H, *J* = 6.0 Hz, $C\underline{H}_3CH(OSi)$), 0.87 (s, 9 H, $(C\underline{H}_3)_3C$), 0.05 (s, 6 H, $(C\underline{H}_3)_2Si$); ^{13}C NMR (90 MHz, $CDCl_3$) δ 166.47 ($\underline{C}OO$), 132.73 (Ar$\underline{C}$), 130.44 (Ar$\underline{C}$), 129.44 (Ar$\underline{C}$), 128.26 (Ar$\underline{C}$), 65.29 ($\underline{C}H(OSi)$), 62.08 ($\underline{C}H_2O$), 38.41 ($\underline{C}H_2CH_2O$), 25.78 ($(\underline{C}H_3)_3C$), 24.04 ($\underline{C}H_3CH(OSi)$), 17.98 ($(CH_3)_3\underline{C}$), -4.43 and -4.97 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) 309 (MH^+, 41); Anal. Calcd for $C_{17}H_{28}O_3Si$: C, 66.19; H, 9.15. Found: C, 65.84; H, 9.20.

***(S)*-3-(*tert*-Butyldimethylsiloxy)butanol (52b).** The method of Seebach and coworkers was used.[111] To a suspension of **51b** (18.0 g, 58.4 mmol) in MeOH (78 mL) and H_2O (12.4 mL) was added a solution of KOH (4.20 g, 74.9 mmol) in H_2O (12.4 mL) over 20 min at 45 °C. The mixture became clear 5 min after the addition and was stirred for 3 h at 45 °C. H_2O (100 mL) was added to the mixture and the aqueous phase was extracted with CH_2Cl_2 (3 x 100 mL). The combined organic phases were washed with saturated solution of $NaHCO_3$ (3 x 100 mL), H_2O (2 x 100 mL), dried over Na_2SO_4, and concentrated *in vacuo* to afford a liquid residue (13.2 g) which was distilled to give **52b** (7.75 g, 65%). Bp 79 °C (1.4 mm Hg); $[\alpha]_D$ 24.2 ° (*c* 0.83, $CHCl_3$); IR (CH_2Cl_2 cast) 3355 (br), 2957 (m), 2950 (s), 2858 (m), 1256 (s) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 4.12 (ddt, 1 H, *J* = 6.8, 6.4, 3.9 Hz, $C\underline{H}(OSi)$), 3.85 and 3.74 (2 x m, 2 H, $C\underline{H}_2OH$), 2.56 (t, 1H, *J* = 5.4 Hz, $O\underline{H}$), 1.80 and 1.65 (2 x m, 2 H, $C\underline{H}_2CH_2OH$), 1.22 (d, 3 H, *J* = 6.4 Hz, $C\underline{H}_3CH(OSi)$), 0.88 (s, 9 H, $(C\underline{H}_3)_3C$), 0.09 (s, 6 H, $(C\underline{H}_3)_2Si$); ^{13}C NMR (90 MHz, $CDCl_3$) δ 68.35 ($\underline{C}H(OSi)$), 60.48 ($\underline{C}H_2OH$), 40.48 ($\underline{C}H_2CH_2OH$), 25.79 ($(\underline{C}H_3)_3C$), 23.42 ($\underline{C}H_3CH(OSi)$), 17.93 ($(CH_3)_3\underline{C}$), -4.37 and -4.96 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) 205 (MH^+, 100); Anal. Calcd for $C_{10}H_{24}O_2Si$: C, 58.77; H, 11.84. Found: C, 58.57; H, 11.61.

***(S)*-[1-^{13}C]-3-(*tert*-Butyldimethylsiloxy)butanol (52c).** A modification of the method of Nicolaou *et al.* was used.[41] To a solution of **41c** (2.07 g, 8.37 mmol) in CH_2Cl_2 (15 mL) was added DIBAL (7.14 g, 50.2 mmol) in CH_2Cl_2 (15 mL) over 15 min at -78 °C. The reaction mixture was stirred for 2 h at -78 °C, and then 30 min at -30 °C, at which point MeOH (4 mL) was added to quench the excess of DIBAL. Then it was diluted with ether (300 mL), and the ether phase was washed with saturated aqueous potassium-sodium tartrate (4 x 100 mL), and brine (3 x 100 mL), dried over Na_2SO_4, and concentrated to give **52c** (1.50 g, 87%). $[\alpha]_D$ +19.64° (*c* 1.1, $CHCl_3$); IR (CH_2Cl_2 cast)

3290 (br), 2959 (m), 2930 (m), 2858 (m), 1255 (m) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.10 (m, 1 H, $C\underline{H}(OSi)$), 4.02 and 3.91 (dm, 1 H, *J* = 40.0 Hz, $C\underline{H}H^{13}CHO$), 3.63 and 3.49 (dm, 1H, *J* = 50.0 Hz, $C\underline{H}H^{13}CHO$), 2.55 (br s, 1 H, $O\underline{H}$), 1.8-1.56 (m, 2 H, $C\underline{H}_2$), 1.18 (d, 3 H, *J* = 5.7 Hz, $C\underline{H}_3CH(OSi)$), 0.88 (s, 9 H, $(C\underline{H}_3)_3C$), 0.08 (s, 6 H, $(C\underline{H}_3)_2Si$); ^{13}C NMR (90 MHz, $CDCl_3$) δ 68.36 ($\underline{C}H(OSi)$), 60.50 (enriched, $^{13}\underline{C}H_2OH$), 40.45 (d, *J* = 36.6 Hz, $\underline{C}H_2$), 25.79 ($(\underline{C}H_3)_3C$), 23.43 ($\underline{C}H_3CH(OSi)$), 17.93 ($(CH_3)_3\underline{C}$), -4.35 and -4.97 ($(\underline{C}H_3)_2Si$); MS (EI) calcd for $^{13}CC_9H_{23}O_2Si$ 204.1051, found 204.1479 (M-H).

***(S)*-[2,3-$^{13}C_2$]-3-(*tert*-Butyldimethylsiloxy)butanol (52d).** The method for the conversion of **41c** to **52c** was used. Thus, **41d** (2.80 g, 11.3 mmol) gave **52d** (1.88 g, 81%). IR (CH_2Cl_2 cast) 3360 (br), 2959 (m), 2929 (m), 2858 (m), 1255 (m) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.29 and 3.94 (dm, 1 H, *J* = 140 Hz, $^{13}C\underline{H}(OSi)$), 3.85 and 3.73 (dm, 2 H, *J* = 48 Hz, $C\underline{H}_2OH$), 1.94 and 1.84 (dm, 1H, *J* = Hz, $^{13}C\underline{H}H$), 1.63 and 1.43 (dm, 1 H, *J* = Hz, $^{13}CH\underline{H}$), 1.21 (dt, 3 H, *J* = 6.3, 4.3 Hz, $C\underline{H}_3{}^{13}CH(OSi)$), 0.90 (s, 9 H, $(C\underline{H}_3)_3C$), 0.10 (s, 6 H, $(C\underline{H}_3)_2Si$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 68.30 (d, *J* = 39.7 Hz, enriched, $^{13}\underline{C}H(OSi)$), 60.50 (t, *J* = 18.9 Hz, $\underline{C}H_2OH$), 40.52 (d, *J* = 39.7 Hz, $^{13}\underline{C}H_2$), 25.80 ($(\underline{C}H_3)_3C$), 23.42 (t, *J* = 19.6 Hz, $\underline{C}H_3{}^{13}CH(OSi)$), 17.95 ($(CH_3)_3\underline{C}$), -4.36 and -4.95 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) 207 (MH^+, 100).

***(S)*-3-(*tert*-Butyldimethylsiloxy)butanal (53b).** The method for conversion of **52c** to **53c** was used. Thus, **52b** (2.75 g, 13.5 mmol) gave **53b** (2.43 g, 89%). Bp 59-60 °C (2 mm Hg); $[\alpha]_D$ +13.9° (*c* 0.90, $CHCl_3$); IR ($CHCl_3$ cast) 1957 (m), 2930 (m), 2897 (m), 2858 (s), 1714 (s), 1472 (m) cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 9.78 (t, 1 H, *J* = 2.4 Hz, $C\underline{H}O$), 4.33 (m, 1 H, $C\underline{H}(OSi)$), 2.48 (m, 2 H, $C\underline{H}_2$), 1.24 (d, 3 H, *J* = 6.4 Hz, $C\underline{H}_3CH(OSi)$), 0.87 (s, 9 H, $(C\underline{H}_3)_3C$), 0.07 (s, 6 H,

$(C\underline{H}_3)_2Si$); ^{13}C NMR (90 MHz, $CDCl_3$) δ 202.14 ($\underline{C}HO$), 64.54 ($\underline{C}H(OSi)$), 52.98 ($\underline{C}H_2$), 25.71 ($(\underline{C}H_3)_3C$), 24.16 ($\underline{C}H_3CH(OSi)$), 17.93 ($(CH_3)_3\underline{C}$), -4.40 and -4.96 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) 203 (MH^+, 59), 220 (MNH_4^+, 25); Anal. Calcd for $C_{10}H_{24}O_2Si$: C, 59.35; H, 10.96. Found: C, 59.65; H, 10.77.

(*S*)-[1-^{13}C]-3-(*tert*-Butyldimethylsiloxy)butanal (53c). The procedure of Swern and coworkers was employed.[117] DMSO (1.10 mL, 14.5 mmol) in CH_2Cl_2 (5 mL) was added to a solution of freshly distilled oxalyl chloride (630 μL, 7.25 mmol) in CH_2Cl_2 (10 mL) over 10 min at -78 °C. After 5 min, the alcohol **52c** (890 mg, 4.24 mmol) in CH_2Cl_2 (7 mL) was added over 10 min. A copious white precipitate was formed. The mixture was stirred for 40 min at -60 °C to -50 °C before triethylamine (2.20 mL, 15.7 mmol) was added. The mixture was warmed to room temperature over 80 min, and H_2O (100 mL) was added. The organic phase was washed with 1N HCl (3 x 50 mL) and H_2O (50 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give a pale yellow residue (1.16 g). The residue was chromatographed on silica (10% ether in pentane, R_f 0.85) to afford **53c** (680 mg, 79%). IR (CH_2Cl_2 cast) 2959 (m), 2930 (m), 2858 (m), 1688 (s), 1258 (m) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 10.04 and 9.56 (dt, 1 H, J = 170, 3.5 Hz, $^{13}C\underline{H}O$), 4.44 (m, 1 H, $C\underline{H}(OSi)$), 2.48 (m, 2 H, $C\underline{H}_2$), 1.19 (d, 3 H, J = 6.4 Hz, $C\underline{H}_3CH(OSi)$), 0.87 (s, 9 H, $(C\underline{H}_3)_3C$), 0.07 (s, 6 H, $(C\underline{H}_3)_2Si$); ^{13}C NMR (90 MHz, $CDCl_3$) δ 204.66 (enriched, $^{13}\underline{C}HO$), 64.54 ($\underline{C}H(OSi)$), 52.95 (d, J = 39.1 Hz, $\underline{C}H_2$), 25.71 ($(\underline{C}H_3)_3C$), 24.13 ($\underline{C}H_3CH(OSi)$), 17.94 ($(CH_3)_3\underline{C}$), -4.39 and -4.96 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) 204 (MH^+, 100).

(*S*)-[2,3-$^{13}C_2$]-3-(*tert*-Butyldimethylsiloxy)butanal (53d). A modification of Nicolaou's procedure was used.[41b] To a solution of the alcohol **52d** (1.84 g, 8.92 mmol) in CH_2Cl_2 (40 mL) was added 4 Å molecular seives (3 g) and PCC (2.60 g, 12.1 mmol). The mixture was stirred for 80 min at room temperature, then poured into ether (300 mL) and filtered. The filtrate was concentrated to give **53d** (1.33 g, 73%). IR (CH_2Cl_2 cast) 2957 (m), 2930 (m), 2858 (m), 1714 (s), 1255 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.83 and 9.77 (dm, 1 H, *J* = 23.0 Hz, $C\underline{H}O$), 4.48 and 4.18 (dm, 1 H, *J* = 140 Hz, $^{13}C\underline{H}(OSi)$), 2.66 and 2.36 (dm, 2 H, *J* = 126 Hz, $^{13}C\underline{H}_2$), 1.24 (dt, 3 H, *J* = 6.0, 4.4 Hz, $C\underline{H}_3{}^{13}CH(OSi)$), 0.88 (s, 9 H, $(C\underline{H}_3)_3C$), 0.07 (s, 6 H, $(C\underline{H}_3)_2Si$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.12 (t, *J* = 20.2 Hz, $\underline{C}HO$), 64.35 (d, *J* = 38.1 Hz, enriched, $^{13}\underline{C}H(OSi)$), 52.98 (d, *J* = 38.0 Hz, $^{13}\underline{C}H_2$), 25.71 ($(\underline{C}H_3)_3C$), 24.17 (t, *J* = 19.6 Hz, $\underline{C}H_3{}^{13}CH(OSi)$), 18.17 ($(CH_3)_3\underline{C}$), -4.39 and -4.96 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) 205 (MH^+, 3.3), 221 (MNH_4^+, 100).

(Carbomethoxymethylene)triphenylphosphorane (54a). The method of Isler *et al.* was used.[119] A mixture of methyl 2-bromoacetate (76.5 g, 500 mmol) and triphenylphosphine (138 g, 525 mmol) in toluene (500 mL) was heated at 120 °C for 1 h. The solvent was removed *in vacuo*, and the resulting solid (205 g) was washed with hexane (200 mL). The residue was dissolved in H_2O (2 L) at 0 °C, and a solution of NaOH (22 g) in H_2O (250 mL) was added over 1 h. The mixture was stirred for 30 min, and then filtered. The white crystals were collected and dried to afford **54b** (145 g, 87%). Mp 169-171 °C; MS (EI) calcd for $C_{21}H_{19}O_2P$ 334.1123, found 334.1103 (M); Anal. Calcd for $C_{21}H_{19}O_2P$: C, 75.43; H, 5.73. Found: C, 75.33; H, 5.90.

[2-^{13}C](Carbomethoxymethylene)triphenylphosphorane (54b). The same method as for preparation of **54a** was used. To obtain methyl [2-^{13}C]bromoacetate, diazomethane was added to a cold (0 °) solution of [2-^{13}C]bromoacetic acid (4.00 g, 28.6 mmol) (isotopic purity 99% ^{13}C) in ether (50 mL) until the mixture retained a pale-yellow coloration. The mixture was allowed to stand for 2 h at room temperature, formic acid (88%, 2 drops) was added to destroy the excess CH_2N_2. Removal of the solvent afforded

pure methyl [2-^{13}C]bromoacetate (4.40 g, 100%). Thus, methyl [2-^{13}C]bromoacetate (4.21 g, 27.3 mmol) gave **54b** (7.92 g, 86%). Mp 163-165 °C; IR (CHCl$_3$ cast) 1618 (s), 1436 (m), 1327 (s), 1104 (s) cm^{-1}; ^{1}H NMR (360 MHz, CDCl$_3$) δ 7.69 (m, 6 H, Ar$\underline{H}$), 7.47 (m, 3 H, Ar$\underline{H}$), 7.41 (m, 6 H, Ar$\underline{H}$), 3.48 (br s, 3 H, C$\underline{H}_3$), 2.88 (br s, 1 H, ^{13}C$\underline{H}$); MS (EI) calcd for ^{13}CC$_{20}$H$_{19}$O$_2$P 335.1158, found 335.1139 (M). For methyl [2-^{13}C]bromoacetate: ^{1}H NMR (360 MHz, CDCl$_3$) δ 3.83 (d, 2 H, J = 156 Hz, ^{13}C$\underline{H}_2$), 3.78 (s, 3 H, C$\underline{H}_3$); ^{13}C NMR (90 MHz, CDCl$_3$) δ 167.66 (d, J = 66.0 Hz, $\underline{C}$O), 53.14 (O$\underline{C}$H$_3$), 25.45 (enriched, ^{13}CH$_2$); MS (EI) calcd for ^{13}CC$_2$H$_5$^{79}BrO$_2$ 152.9506, found 152.9497 (M); calcd for ^{13}CC$_2$H$_5$^{81}BrO$_2$ 154.9486, found 154.9479 (M+2).

[1,2-^{13}C$_2$, 1-^{14}C](Carbomethoxymethylene)triphenylphosphorane (54c). The same method as for preparation of **54a** was used. Thus, a mixture of methyl [1,2-^{13}C$_2$]bromoacetate and methyl [1-^{14}C]bromoacetate (1.36 g, 8.77 mmol) gave **54c** (2.70 g, 92%).

Methyl (*S*)-[2,3-^{13}C$_2$]-5-(*tert*-Butyldimethylsiloxy)hex-2-enoate (55d). The method of Seebach and coworkers was used.[111] The aldehyde **53c** (930 mg, 4.57 mmol) and the Wittig reagent **54b** (1.66 g, 4.95 mmol) were dissolved in dry benzene (35 mL), and the resulting solution was heated to reflux at 85 °C for 8 h. The mixture was cooled to room temperature and stirred overnight. The solvent was removed by distillation and the residue was washed thoroughly with pentane. Upon concentration of the filtrate, a yellow residue (solid + liquid) (1.70 g) was obtained. The residue was purified on flash silica (10 x 4.5 cm) (10% ether in pentane, R_f 0.66) to yield **55d** (730 mg, 61%) as a mixture of inseparable isomers (Z/E = 4.5/95.5). $[\alpha]_D$ +8.3° (c 0.95, CHCl$_3$); IR (CHCl$_3$ cast) 2953 (m), 2930 (m), 2890 (m), 2857 (m), 1726 (s), 1605 (m), 1313 (m), 1258 (m), 1220 (m), 1171 (m), 1130 (m), 1066 (m), 1057 (m), 1005 (m), 836 (m), 775 (m) cm^{-1}; ^{1}H NMR (360 MHz, CDCl$_3$) δ 7.17 and 6.76 (dddt, 1H, J = 156, 15.6, 7.5, 1.95 Hz, $^{13}\mathrm{C\underline{H}}={}^{13}\mathrm{CHCO}$), 6.07 and 5.62 (br dt, 1H, *J* = 162, 15.6 Hz, $^{13}\mathrm{CH}={}^{13}\mathrm{C\underline{H}CO}$), 3.94 (m, 1H, $\mathrm{C\underline{H}OSi}$), 3.76 (s, 3H, $\mathrm{O\underline{C}H_3}$), 2.34 (m, 2H, $\mathrm{C\underline{H_2}{}^{13}CH}$), 1.15 (d, 3H, *J* = 5.4 Hz, $\mathrm{C\underline{H}_3CH(OSi)}$), 0.87 (s, 9H, $\mathrm{(C\underline{H}_3)_3C}$), 0.06 (s, 6H, $\mathrm{(CH_3)_2Si}$); ^{13}C NMR (90 MHz, $\mathrm{CDCl_3}$) δ 166.60 (d, *J* = 74.46 Hz, $\mathrm{\underline{C}O}$), 146.03 (d, *J* = 69.58 Hz, $^{13}\mathrm{\underline{C}H}={}^{13}\mathrm{CHCO}$), 122.72 (d, *J* = 69.58 Hz, $^{13}\mathrm{CH}={}^{13}\mathrm{\underline{C}HCO}$), 67.51 ($\mathrm{\underline{C}H(OSi)}$), 51.15 ($\mathrm{O\underline{C}H_3}$), 42.34 (d, *J* = 41.51 Hz, $\mathrm{\underline{C}H_2{}^{13}CH}$), 25.69 ($\mathrm{(\underline{C}H_3)_3C}$), 23.62 ($\mathrm{\underline{C}H_3CH(OSi)C}$), 17.93 ($\mathrm{(CH_3)_3\underline{C}}$), -4.66 and -4.95 ($\mathrm{(\underline{C}H_3)_2Si}$); MS (CI, $\mathrm{NH_3}$) 261 ($\mathrm{M^+}$, 50), 262($\mathrm{MH^+}$, 9), 278 ($\mathrm{MNH_4^+}$, 100).

For unlabeled racemic material (**55a**): IR ($\mathrm{CHCl_3}$ cast) 2954 (m), 2930 (m), 2890(m), 2858, (m), 1728 (s), 1680 (m) $\mathrm{cm^{-1}}$; ^{1}H NMR (360 MHz, $\mathrm{CDCl_3}$) δ 6.96 (ddt, 1H, *J* =15.6, 6.8, 1.5 Hz, $\mathrm{C\underline{H}=CHCO}$), 5.84 (dt, 1H, *J* = 15.6, 1.5 Hz, $\mathrm{CH=C\underline{H}CO}$), 3.97 (dq, 1 H, *J* = 5.9, 5.9 Hz, $\mathrm{C\underline{H}OSi}$), 3.74 (s, 3 H, $\mathrm{OC\underline{H}_3}$), 2.34 (ddd, 2H, *J* = 6.8, 5.9, 1.5 Hz, $\mathrm{C\underline{H}_2CH=CH}$), 1.18 (dt, 3H, *J* = 5.9, 1.5 Hz, $\mathrm{C\underline{H}_3CH(OSi)CH_2}$), 0.90 (s, 9H, $\mathrm{(CH_3)_3C}$), 0.06 (s, 6H, $\mathrm{(CH_3)_2Si}$); ^{13}C NMR (90 MHz, $\mathrm{CDCl_3}$) δ 166.73 ($\mathrm{\underline{C}O}$), 146.21 ($\mathrm{\underline{C}H=CHCO}$), 122.76 ($\mathrm{CH=\underline{C}HCO}$), 67.54 ($\mathrm{\underline{C}H(OSi)}$), 51.25 ($\mathrm{O\underline{C}H_2}$), 42.40 ($\mathrm{\underline{C}H_2CH=CH}$), 25.74 ($\mathrm{(\underline{C}H_3)_3C}$), 23.66 ($\mathrm{\underline{C}H_3CH(OSi)CH_2}$), 18.00 ($\mathrm{(CH_3)_3\underline{C}}$), -4.62 and -4.89 ($\mathrm{(\underline{C}H_3)_2Si}$); MS (CI, $\mathrm{NH_3}$) 276 ($\mathrm{MH^+}$, 100); Anal. Calcd for $\mathrm{C_{13}H_{26}O_3Si}$: C, 60.42; H, 10.14. Found: C, 60.18; H, 10.00.

Methyl [1,2-^{13}C$_2$,1-^{14}C]-5-(*tert*-Butyldimethylsiloxy)hex-2-enoate (55e). The method for conversion of **53c** to **55d** was used. Thus, 3-(*tert*-butyldimethyl siloxy)butanal (**53a**) (650 mg, 3.21 mmol) and [1,2-^{13}C$_2$](carbomethoxy methylene)triphenylphosphorane (**54c**) (800 mg, 2.38 mmol) afforded **55e** (430 mg, 70%). ^{1}H NMR (400 MHz, $\mathrm{CDCl_3}$) δ 6.95 (m, 1 H, $\mathrm{C\underline{H}={}^{13}CH}$), 6.03 and 5.63 (m, 1 H, $\mathrm{CH={}^{13}C\underline{H}}$), 3.90 (m, 1 H, $\mathrm{C\underline{H}(OSi)}$), 3.72 (d, 3 H, *J* = 4.0 Hz, $\mathrm{OC\underline{H}_3}$), 2.40 (m, 2 H, $\mathrm{C\underline{H}_2CH={}^{13}CH}$), 1.16 (d, 3 H, *J* = 6.2 Hz, $\mathrm{C\underline{H}_3CH(OSi)}$), 0.88 (s, 9H, $\mathrm{(CH_3)_3C}$), 0.06 (s, 6H, $\mathrm{(CH_3)_2Si}$); ^{13}C NMR (75 MHz, $\mathrm{CDCl_3}$) δ 166.88 (d, *J* = 75.4 Hz, enriched,

$^{13/14}\underline{C}O$), 146.25 (d, *J* = 70.5 Hz, $\underline{C}H{=}^{13}CH$), 122.75 (d, *J* = 75.4 Hz, enriched, $CH{=}^{13}\underline{C}H$), 67.65 ($\underline{C}H(OSi)$), 51.38 ($O\underline{C}H_3$), 42.47 (d, *J* = 6.1 Hz, $\underline{C}H_2CH{=}^{13}CH$), 25.75 ($(\underline{C}H_3)_3C$), 23.76 ($\underline{C}H_3CH(OSi)$), 18.10 ($(CH_3)_3\underline{C}$), -4.52 and -4.80 ($(\underline{C}H_3)_2Si$);

***(S)*-[2,3-$^{13}C_2$]-5-(*tert*-Butyldimethylsiloxy)hex-2-enoic Acid (56d).** The procedure of Seebach and coworkers was adapted.[111] To a solution of the methyl ester **55d** (720 mg, 2.77 mmol) in THF (19.7 mL) and H_2O (4.90 mL) was added aqueous NaOH (3.3 mL, 3.3 mmol) over 15 min. Then the reaction mixture was heated to 30 °C and stirred for 3 h. Half of the THF was removed *in vacuo*, the remaining mixture was stirred at 30 °C for 20 h, and eventually became a clear solution. The solvent was removed, and the aqueous phase was acidified to pH 2 by 2N HCl, and extracted with $CHCl_3$ (3 x 50 mL). The combined organic phase was dried over Na_2SO_4 and concentrated to yield pure **56d** (644 mg, 95%). $[\alpha]_D$ +8.9° (*c* 0.59, $CHCl_3$); IR ($CHCl_3$ cast) 2400-3400 (br), 2956 (m), 2930 (m), 2885(m), 2858, (m), 1695 (s), 1601 (m), 1418(m), 1312 (m), 1291 (m), 1276 (m), 1257 (m), 1131 (m), 1085 (m), 1004 (m), 836 (m), 775 (m) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.31 and 6.86 (dddt, 1 H, *J* = 156, 15.6, 7.7, 1.95 Hz, $^{13}C\underline{H}{=}^{13}CHCO$), 6.08 and 5.63 (br dt, 1 H, *J* = 163, 15.6 Hz, $^{13}CH{=}^{13}C\underline{H}CO$), 3.94 (dq, 1 H, *J* = 5.9, 5.9 Hz, $C\underline{H}OSi$), 2.36 (m, 2 H, $C\underline{H}_2{}^{13}CH$), 1.20 (d, 3 H, *J* = 5.9 Hz, $C\underline{H}_3CH(OSi)$), 0.89 (s, 9 H, $(CH_3)_3C$), 0.06 (s, 6 H, $(CH_3)_2Si$); ^{13}C NMR (90 MHz, $CDCl_3$) δ 171.76 (d, *J* = 72.0 Hz, $\underline{C}O$), 148.90 (d, *J* = 69.6 Hz, $^{13}\underline{C}H{=}^{13}CHCO$), 122.60 (d, *J* = 69.6 Hz, $^{13}CH{=}^{13}\underline{C}HCO$), 67.51 ($\underline{C}H(OSi)$), 42.48 (d, J = 42.73 Hz, $\underline{C}H_2{}^{13}CH$), 25.78 ($(\underline{C}H_3)_3C$), 23.75 ($\underline{C}H_3CH(OSi)CH_2$), 18.04 ($(CH_3)_3\underline{C}$), -4.57 and -4.84 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) 247 (MH^+, 52), 264 (MNH_4^+, 95).

For unlabeled racemic material (**56a**): IR ($CHCl_3$ cast) 3400-2400 (br), 2956 (m), 2930 (m), 2885(m), 2858, (m), 1699 (s), 1654 (m) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.08 (dt, 1H, *J* = 15.6, 7.7 Hz, $C\underline{H}{=}CHCO$), 5.85 (dt, 1H, *J* = 15.6, 1.4 Hz, CH=C$\underline{H}$CO), 3.95 (m, 1 H, C$\underline{H}$OSi), 2.35 (m, 2H, C$\underline{H}_2$CH=CH), 1.18 (dt, 3H, J = 6.2 Hz, C$\underline{H}_3$CH(OSi)), 0.87 (s, 9H, $(CH_3)_3C$), 0.06 (s, 6H, $(CH_3)_2Si$); ^{13}C NMR (90 MHz, $CDCl_3$) δ 171.50 ($\underline{C}$O), 149.00 ($\underline{C}$H=CHCO), 122.53 (CH=$\underline{C}$HCO), 67.49 ($\underline{C}$H(OSi)), 42.49 ($\underline{C}H_2$CH=CH), 25.77 (($\underline{C}H_3)_3$C), 23.78 ($\underline{C}H_3$CH(OSi)), 18.03 (($CH_3)_3\underline{C}$), -4.58 and -4.85 (($\underline{C}H_3)_2$Si); MS (CI, NH_3) 276 (MH$^+$, 100); Anal. Calcd for $C_{13}H_{26}O_3Si$: C, 58.97; H, 9.90. Found: C, 58.98; H, 9.52.

[1,2-$^{13}C_2$,1-^{14}C]-5-(*tert*-Butyldimethylsiloxy)hex-2-enoic Acid (56e). The method for conversion of **55d** to **56d** was used. Thus, **55e** (430 mg, 1.65 mmol) afforded **56e** (290 mg, 71%). IR ($CHCl_3$ cast) 3400-2400 (br), 2957 (m), 2929 (m), 2857 (m), 1660 (s), 1622 (m), 1257 (m) cm^{-1}; ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.23 (d, J = 72.4 Hz, enriched, $^{13/14}\underline{C}$O), 149.05 (d, J = 69.9 Hz, $\underline{C}$H=^{13}CH), 122.50 (d, J = 72.2 Hz, enriched, CH=$^{13}\underline{C}$H), 67.54 ($\underline{C}$H(OSi)), 42.57 (d, J = 6.2 Hz, $\underline{C}H_2$CH=^{13}CH), 25.85 (($\underline{C}H_3)_3$C), 23.85 ($\underline{C}H_3$CH(OSi)), 18.11 (($CH_3)_3\underline{C}$), -4.57 and -4.85 (($\underline{C}H_3)_2$Si); MS (CI, NH_3) 247 (MH$^+$, 20), 264 (MNH_4^+, 100).

NAC (*S*)-[2,3-$^{13}C_2$]-5-(*tert*-Butyldimethylsiloxy)hex-2-enoate (57d). A modification of the method used by Parker was followed.[120] To a solution of the acid **56d** (624 mg, 2.53 mmol) in CH_2Cl_2 (5 mL) were added *N*-acetyl cysteamine **45** (320 mg, 2.69 mmol) in CH_2Cl_2 (5 mL), DCC (560 mg, 2.71 mmol), and 4-dimethylaminopyridine (10 mg) in CH_2Cl_2 (4.2 mL) simultaneously over 5 min at -10 °C. The solution became a cloudy white and was stirred overnight at room temperature. The mixture was concentrated to afford a yellow oil, which was purified by flash chromatography (silica, 2 x 14 cm, EtOAc, R_f 0.37) to give **57d** (815 mg, 93%). $[\alpha]_D$ +7.5° (c 0.51, $CHCl_3$); IR ($CHCl_3$ cast) 3279 (br), 2956 (m), 2928 (m), 2855 (m), 1658 (s), 1630 (m), 1581 (m), 1559 (m), 1254 (m), 1004 (m), 895 (m), 775 (m) cm^{-1}; ^{1}H

NMR (360 MHz, $CDCl_3$) δ 7.14 and 6.73 (ddt, 1 H, *J* = 136, 15.6, 7.8 Hz, $^{13}C\underline{H}={}^{13}CHCOS$), 6.37 and 5.92 (br dt, 1 H, *J* = 154, 15.6 Hz, $^{13}CH={}^{13}C\underline{H}COS$), 5.88 (br s, 1 H, $N\underline{H}$), 3.96 (m, 1 H, $C\underline{H}OSi$), 3.47 (dt, 2 H, *J* = 6.4, 5.5 Hz, $C\underline{H}_2NH$), 3.11 (t, 2 H, *J* = 6.4 Hz, $SC\underline{H}_2$), 2.34 (m, 2 H, $C\underline{H}_2{}^{13}CH$), 1.98 (s, 3 H, $COC\underline{H}_3$), 1.17 (d, 3 H, *J* = 6.4 Hz, $C\underline{H}_3CH(OSi)CH_2$), 0.88 (s, 9 H, $(CH_3)_3C$), 0.07 (s, 6 H, $(CH_3)_2Si$); ^{13}C NMR (90 MHz, $CDCl_3$) δ 190.15 (d, *J* = 61.0 Hz, $\underline{C}OS$), 170.23 ($\underline{C}OCH_3$), 143.43 (d, *J* = 70.0 Hz, $^{13}\underline{C}H={}^{13}CHCOS$), 130.22 (d, *J* = 70.0 Hz, $^{13}CH={}^{13}\underline{C}HCOS$), 67.43 ($\underline{C}H(OSi)$), 42.32 (d, *J* = 41.5 Hz, $\underline{C}H_2{}^{13}CH$), 39.79 ($CO\underline{C}H_3$), 28.13 ($S\underline{C}H_2$), 25.74 ($(\underline{C}H_3)_3C$), 23.86 ($\underline{C}H_3CH(OSi)CH_2$), 23.15 ($\underline{C}H_2NH$), 17.98 ($(CH_3)_3\underline{C}$), -4.55 and -4.85 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) 348 (MH^+, 100), 365 (MNH_4^+, 51); MS (EI) calcd for $^{13}C_2C_{14}H_{31}NO_3SSi$ 347.1861, found 347.1834 (M).

For unlabeled racemic material (**57a**): IR ($CHCl_3$ cast) 3288 (br), 2955 (m), 2929 (m), 2858(m), 1668 (s), 1637 (m), 1552 (m) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 6.91 (dt, 1H, *J* = 15.62, 7.7 Hz, $C\underline{H}=CHCOS$), 6.13 (br d, 1H, *J* = 15.6 Hz, $CH=C\underline{H}COS$), 5.95 (br s, 1H, $N\underline{H}$), 3.93 (m, 1H, $C\underline{H}OSi$), 3.44 (dt, 2H, *J* = 6.4, 5.5 Hz, $C\underline{H}_2NH$), 3.07 (t, 2H, *J* = 6.4 Hz, $SC\underline{H}_2$), 2.30 (m, 2H, $C\underline{H}_2CH=CH$), 1.95 (s, 3H, $COC\underline{H}_3$), 1.15 (d, 3H, *J* = 6.2 Hz, $C\underline{H}_3CH(OSi)CH_2$), 0.88 (s, 9H, $(CH_3)_3C$), 0.07 (s, 6H, $(CH_3)_2Si$); ^{13}C NMR (90 MHz, $CDCl_3$) δ 190.24 ($\underline{C}OS$), 170.23 ($\underline{C}OCH_3$), 143.53 ($\underline{C}H=CHCOS$), 130.15 ($CH=\underline{C}HCOS$), 67.45 ($\underline{C}H(OSi)$), 42.39 ($\underline{C}H_2CH=CH$), 39.84 ($CO\underline{C}H_3$), 28.15 ($S\underline{C}H_2$), 25.76 ($(\underline{C}H_3)_3C$), 23.67 ($\underline{C}H_3CH(OSi)CH_2$), 22.83 ($\underline{C}H_2NH$), 18.00 ($(CH_3)_3\underline{C}$), -4.55 and -4.84 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) 346 (MH^+, 57); Anal. Calcd for $C_{16}H_{31}NO_3SSi$: C, 55.61; H, 9.04; N, 4.05. Found: C, 55.21; H, 9.00; N, 4.23.

NAC [1,2-$^{13}C_2$,1-^{14}C]-5-(*tert*-Butyldimethylsiloxy)hex-2-enoate (57e). The method for conversion of **56d** to **57d** was used. Thus, **56e** (283 mg, 1.15 mmol) afforded **57e** (157 mg, 39%). IR ($CHCl_3$ cast) 3240 (br), 2928 (m), 1640 (s), 1603 (m), 1554 (m) cm^{-1}; ^{1}H NMR (200 MHz, CDCl$_3$) δ 6.94 (dt, 1 H, *J* = 15.6, 7.7 Hz, $C\underline{H}{=}^{13}CH$), 6.56 and 5.78 (ddm, 1 H, *J* = 160, 15.6 Hz, $CH{=}^{13}C\underline{H}$), 5.90 (br s, 1 H, $N\underline{H}$), 3.93 (m, 1 H, $C\underline{H}(OSi)$), 3.44 (q, 2 H, *J* = 6.2 Hz, $C\underline{H}_2NH$), 3.10 (t, 2 H, *J* = 6.3 Hz, $SC\underline{H}_2$), 2.32 (m, 2 H, $C\underline{H}_2CH{=}^{13}CH$), 1.97 (s, 3 H, $COC\underline{H}_3$), 1.17 (d, 3 H, *J* = 6.2 Hz, $C\underline{H}_3CH(OSi)$), 0.88 (s, 9 H, $(CH_3)_3C$), 0.07 (s, 6 H, $(CH_3)_2Si$); ^{13}C NMR (90 MHz, CDCl$_3$) δ 190.24 (d, *J* = 61.8 Hz, enriched, $^{13}\underline{C}OS$), 170.21 ($\underline{C}OCH_3$), 143.56 (d, *J* = 70.3 Hz, $\underline{C}H{=}^{13}CH$), 130.12 (d, *J* = 61.8 Hz, enriched, $CH{=}^{13}\underline{C}H$), 67.52 ($\underline{C}H(OSi)$), 42.45 (d, *J* = 6.0 Hz, $\underline{C}H_2CH{=}^{13}CH$), 39.88 ($CO\underline{C}H_3$), 28.22 ($S\underline{C}H_2$), 25.79 ($(\underline{C}H_3)_3C$), 23.88 ($\underline{C}H_3CH(OSi)$), 23.20 ($\underline{C}H_2NH$), 18.10 ($(CH_3)_3\underline{C}$), -4.51 and -4.80 ($(\underline{C}H_3)_2Si$); MS (CI, NH$_3$) 348 (MH$^+$, 67), 365 (MNH$_4^+$, 100).

5-(*tert*-Butyldimethylsiloxy)hex-2-enal (62). The method of Seebach and coworkers was used.[111] A mixture of methyl 5-(*tert*-butyldimethylsiloxy)hex-2-enol **70** (461 mg, 2.00 mmol) and active manganese dioxide[124] (870 mg, 20.0 mmol) in CH$_2$Cl$_2$ (10 mL) was stirred for 12 h and filtered. The filtrate was concentrated to give the known[111] aldehyde **62** (370 mg, 81%). IR (CHCl$_3$ cast) 2956 (s), 2930 (s), 2930 (s), 2858 (s), 1728 (s), 1697 (m), 1473 (m) cm^{-1}; ^{1}H NMR (200 MHz, CDCl$_3$) δ 9.62 (d, 1 H, *J* = 8.0 Hz, $C\underline{H}O$), 6.98 (m, 1 H, $C\underline{H}{=}CHCHO$), 5.84 (m, 1 H, $CH{=}C\underline{H}CHO$), 3.92 (m, 1 H, $C\underline{H}(OSi)$), 2.30 (m, 2 H, $C\underline{H}_2CH{=}CH$), 1.13 (d, 3 H, *J* = 6.0 Hz, $C\underline{H}_3CH(OSi)$), 0.85 (s, 9 H, $(C\underline{H}_3)_3C$), 0.04 (s, 6 H, $(C\underline{H}_3)_2Si$); MS (CI, NH$_3$) 246 (MNH$_4^+$, 46).

(Carbethoxymethylene)triphenylphosphorane (68a). The same method as for the preparation of **54a** was used. Thus, ethyl bromoacetate (16.7 g, 100 mmol) gave **68a** (31.9 g, 92%). Mp 128-130 °C: MS (EI) calcd for $C_{22}H_{21}O_2P$ 348.1279, found 348.1267 (M)

[1-^{13}C](Carbethoxymethylene)triphenylphosphorane (68b). The same method as for preparation of **54a** was used. Thus, ethyl [1-^{13}C]bromoacetate (2.00 g, 11.9 mmol) (isotopic purity 99% ^{13}C) gave **68b** (3.38 g, 81%). Mp 123-125 °C; IR (CHCl$_3$ cast) 1600 (s), 1579 (m), 1437 (m), 1322 (m), 1102 (m) cm^{-1}; MS (EI) calcd for ^{13}CC$_{20}$H$_{19}$O$_2$P 349.1315, found 349.1301 (M).

Ethyl *(S)*-[1-^{13}C]-5-(*tert*-Butyldimethylsiloxy)hex-2-enoate (69c). A similar procedure to that for the conversion of **53c** to **55d** was used. Thus, the aldehyde **53c** (2.24 g, 11.1 mmol) afforded **69c** (1.85 g, 70%), after column chromatography (silica, 10% EtOAc in hexanes, R_f 0.55). IR (CH$_2$Cl$_2$ cast) 2957 (m), 2930 (m), 2857 (m), 1685 (s), 1651 (m) cm^{-1}; ^{1}H NMR (360 MHz, CDCl$_3$) δ 6.92 (dt, 1 H, *J* = 15.6, 7.7 Hz, C$\underline{H}$=CH^{13}CO), 5.81 (dt, 1 H, *J* = 15.6, 1.5 Hz, CH=C$\underline{H}^{13}$CO), 4.15 (m, 2 H,OC$\underline{H}_2$), 3.88 (m, 1 H, C$\underline{H}$(OSi)), 2.28 (m, 2 H, C$\underline{H}_2$CH=CH), 1.23 (t, 3 H, *J* = 7.1 Hz, CH$_2$C$\underline{H}_3$), 1.13 (d, 3 H, *J* = 6.4 Hz, C$\underline{H}_3$CH(OSi)), 0.87 (s, 9 H, (C$\underline{H}_3$)$_3$C), 0.07 (s, 6 H, (C$\underline{H}_3$)$_2$Si); ^{13}C NMR (90 MHz, CDCl$_3$) δ 166.43 (enriched, $\underline{C}$OO), 145.99 ($\underline{C}$H=CH^{13}CO), 123.18 (d, *J* = 74.5 Hz, CH=$\underline{C}$H^{13}CO), 67.66 ($\underline{C}$H(OSi)), 60.11 (O$\underline{C}$H$_2$), 42.43 (d, *J* = 7.32 Hz, $\underline{C}$H$_2$CH=CH), 25.79 (($\underline{C}$H$_3$)$_3$C), 23.77 ($\underline{C}$H$_3$CH(OSi)), 18.06 ((CH$_3$)$_3\underline{C}$), 14.26 (CH$_2\underline{C}$H$_3$), -4.54 and -4.85 (($\underline{C}$H$_3$)$_2$Si); MS (CI, NH$_3$) 274 (MH$^+$, 55), 291 (MNH$_4^+$, 100).

For unlabeled racemic material (**69a**): IR (CHCl$_3$ cast) 2957 (m), 2930 (m), 2887(m), 2855, (m), 1724 (s), 1659 (m) cm^{-1}; ^{1}H NMR (360 MHz, CDCl$_3$) δ 6.89 (dt, 1H, *J* = 15.6, 7.8 Hz, C$\underline{H}$=CHCO), 5.83 (dt, 1H, *J* = 15.62, 1.5 Hz, CH=C$\underline{H}$CO), 4.19 (q, 2H, *J* = 7.3 Hz, OC$\underline{H}_2$), 3.92 (ddt, 1 H, *J* = 5.9, 5.9, 1.5 Hz, C$\underline{H}$OSi), 2.36 (m, 2H, C$\underline{H}_2$CH=CH), 1.28 (t, 3 H, *J* = 7.3 Hz, CH$_2$C$\underline{H}_3$), 1.18 (d, 3H, *J* = 5.9 Hz, C$\underline{H}_3$CH(OSi)), 0.90 (s, 9H, (CH$_3$)$_3$C), 0.06 (s, 6H, (CH$_3$)$_2$Si); ^{13}C NMR (90 MHz, CDCl$_3$) δ 166.40 ($\underline{C}$O), 145.98 ($\underline{C}$H=CHCO), 123.21 (CH=$\underline{C}$HCO), 67.64 ($\underline{C}$H(OSi)), 60.09 (O$\underline{C}$H$_2$), 42.43 ($\underline{C}$H$_2$CH=CH), 25.79 (($\underline{C}$H$_3$)$_3$C), 23.76 ($\underline{C}$H$_3$CH(OSi)CH$_2$), 18.05 ($(CH_3)_3\underline{C}$), 14.24 ($CH_2\underline{C}H_3$), -4.56 and -4.85 ($(\underline{C}H_3)_2Si$); MS (EI) calcd for $C_{10}H_{19}O_3Si$ 215.1104, found 215.1104 (M-C_4H_9).

Ethyl *(S)*-[4,5-$^{13}C_2$]-5-(*tert*-Butyldimethylsiloxy)hex-2-enoate (69d). The method for the conversion of **53c** to **55d** was used. Thus, the aldehyde **53d** (1.32 g, 6.46 mmol) afforded **69d** (1.06 g, 60%). IR (CH_2Cl_2 cast) 2958 (m), 2929 (m), 2858 (m), 1724 (s), 1655 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.95 (ddt, 1 H, *J* = 15.6 Hz, $C\underline{H}$=CHCO), 5.83 (dt, 1 H, *J* = 15.6 Hz, CH=$C\underline{H}$CO), 4.19 (q, 2 H, *J* = 7.1 Hz, $OC\underline{H}_2$), 4.09 and 3.74 (dm, 1 H, *J* = 140 Hz, $^{13}C\underline{H}(OSi)$), 2.47 and 2.15 (dm, 2 H, *J* = 128 Hz, $^{13}C\underline{H}_2$), 1.28 (t, 3 H, *J* = 7.1 Hz, $CH_2C\underline{H}_3$), 1.16 (dt, 3 H, *J* = 6.0, 4.4 Hz, $C\underline{H}_3{}^{13}CH(OSi)$), 0.87 (s, 9 H, $(C\underline{H}_3)_3C$), 0.07 (s, 6 H, $(C\underline{H}_3)_2Si$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.43 (d, *J* = 6.0 Hz, $\underline{C}OO$), 146.04 (t, *J* = 21.5 Hz, $\underline{C}H$=CHCO), 123.20 (d, *J* = 3.2 Hz, CH=$\underline{C}$HCO), 67.65 (d, *J* = 38.6 Hz, enriched, $^{13}\underline{C}H(OSi)$), 60.12 ($O\underline{C}H_2$), 42.43 (d, *J* = 38.6 Hz, enriched, $^{13}\underline{C}H_2$), 25.79 ($(\underline{C}H_3)_3C$), 23.77 (t, *J* = 19.7 Hz, $\underline{C}H_3{}^{13}CH(OSi)$), 18.07 ($(CH_3)_3\underline{C}$), 14.25 ($CH_2\underline{C}H_3$), -4.55 and -4.85 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) 275 (MH^+, 85), 292 (MNH_4^+, 100).

5-(*tert*-Butyldimethylsiloxy)hex-2-enol (70). The method for the conversion of **41c** to **52c** was used. Thus, methyl 5-(*tert*-butyldimethylsiloxy)hex-2-enoate (**55a**) (2.78 g, 10.8 mmol) gave the known[111] compound **70** (2.30 g, 93%). IR ($CHCl_3$ cast) 3340 (br), 2956 (s), 2930 (s), 2858 (s), 1470 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.66-5.58 (m, 2 H, $C\underline{H}$=$C\underline{H}$), 4.04 (m, 2 H, $C\underline{H}_2OH$), 3.79 (m, 1 H, $C\underline{H}(OSi)$), 2.20-2.05 (m, 2 H, $C\underline{H}_2$CH=CH), 1.09 (d, 3 H, *J* = 6.0 Hz, $C\underline{H}_3CH(OSi)$), 0.85 (s, 9 H, $(C\underline{H}_3)_3C$), 0.04 (s, 6 H, $(C\underline{H}_3)_2Si$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 131.29 and 129.48 ($\underline{C}H$=$\underline{C}H$), 68.45 ($\underline{C}H(OSi)$), 63.55 ($\underline{C}H_2OH$), 42.51, ($\underline{C}$HCH=CH), 25.86 ($(\underline{C}H_3)_3C$), 23.26 ($\underline{C}H_3CH(OSi)$), 18.14 ($(CH_3)_3\underline{C}$), -4.53 and -4.72 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) 231(MH^+, 68), 248 (MNH_4^+, 100).

1,1,4,4-Tetramethoxy-2-butene (71). The procedure of Makin *et al.* was used.[122b] To a cold (-45 °C) solution of furan (34.0 g, 500 mmol) in MeOH (250 mL) was added liquid bromine (79.9 g, 500 mmol) in MeOH (200 mL) over 40 min. The reaction mixture was stirred for 1.5 h between -10 to -5 °C and cooled to -40 °C again. The mixture was neutralized with gaseous ammonia to pH 8, the ammonium bromide precipitate generated was then filtered and washed with ether. The filtrate was concentrated to give an orange-red liquid (79.5 g), which was distilled to afford the known[122b] compound **71** (68.7 g, 78%). Bp 92-94 °C (6.5 mm Hg); IR (CHCl$_3$ cast) 2989 (m), 2938 (s), 2908 (m), 2830 (s), 1468 (m), 1445 (m), 1056 (s) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.82 (dd, 2 H, *J* = 2.2, 1.2 Hz), 4.84 (dd, 2 H, *J* = 2.2, 1.1 Hz), 3.33 (s, 12 H, 4 x OC$\underline{H}_3$); ^{13}C NMR (75 MHz, CDCl$_3$) δ 130.93 ($\underline{C}$H=$\underline{C}$H), 101.94 (2 x $\underline{C}$H(OMe), 52.69 (4 x O$\underline{C}$H$_3$); MS (EI) calcd for $C_7H_{13}O_3$ 145.0865, found 145.0869 (M-OCH$_3$).

4,4-Dimethoxycrotonaldehyde (72). The procedure of Yanovskaya *et al.* was followed.[122c] A mixture of the bisacetal **71** (68.0 g, 386 mmol), 6% phosphoric acid (3.9 mL), and H$_2$O (3.4 mL) was heated for 80 min at 100 °C. During this process, MeOH (ca. 8 mL) was distilled out of the mixture. The reaction mixture was then distilled to produce the known[122c] aldehyde **72** (50.0 g, 72%) (It was contaminated with ca. 22% starting bisacetal **71** as showed by ^{1}H NMR). Bp 60-61 °C (1 mm Hg); IR (CHCl$_3$ cast) 2993 (m), 2940 (m), 2911 (m), 2833 (m), 1726 (m), 1697 (s), 1468 (m), 1445 (m), 1058 (s) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$) δ 9.63 (d, 1 H, *J* = 8.1 Hz, C$\underline{H}$O), 5.63 (dd, 1 H, *J* = 15.9, 4.0 Hz, C$\underline{H}$=CHCHO), 6.37 (ddd, 1 H, *J* = 15.9, 8.0, 1.4 Hz, C$\underline{H}$CHO), 5.06 (dd, 1 H, *J* = 4.0, 1.4 Hz, (OMe)$_2$C$\underline{H}$), 3.47 (s, 6 H, 2 x OC$\underline{H}_3$); ^{13}C NMR (75 MHz, CDCl$_3$) δ 193.08 ($\underline{C}$HO), 150.33 ($\underline{C}$H=CHCHO), 134.19 (CH=$\underline{C}$HCHO), 100.46 ($\underline{C}$H(OMe), 52.98 (2 x O$\underline{C}$H$_3$); MS (EI) calcd for $C_5H_7O_2$ 99.0446, found 99.0446 (M-OCH$_3$).

Methyl 6,6-Dimethoxy-2,4-hexadienoate (73). A modification of the method of De Koning *et al.* was used.[122a] A mixture of **72** (39.5 g, crude, ca. 237 mmol) and the Wittig reagent (**54a**) (79.5 g, 238 mmol) in toluene (225 mL) was heated to reflux for 1 h. The solvent was removed *in vacuo*, the resulting residue was dissolved in hexanes (200 mL) and filtered. The filtrate was concentrated and the resulting brown liquid was distilled to afford the known[122a] compound **73** (28.0 g, 64%). Bp 82-84 °C (0.1 mm Hg); IR ($CHCl_3$ cast) 2953 (m), 2833 (m), 1722 (s), 1639 (m), 1054 (m) cm^{-1}; MS (EI) calcd for $C_9H_{14}O_4$ 186.0892, found 186.0890 (M).

Methyl 6-Oxo-2,4-hexadienoate(75). The procedure of Koning *et al.* was followed.[122a] A mixture of the acetal (**73**) (27.5 g, 148 mmol) and sodium acetate (13.4 g, 163 mmol) in acetic acid (135 mL) and H_2O (10 mL) was heated for 2 h at 100 °C. The mixture was poured onto crushed ice (500 mL), and the resulting aqueous solution was extracted with ether (3 x 200 mL). The combined organic phases were dried over Na_2SO_4 and concentrated to give **75** (12.6 g, 61%) as a yellow solid. Mp 69.0-71.0 °C; IR (acetone cast) 1725 (m), 1678 (s), 1326 (m), 1233 (s), 1010 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 9.68 (d, 1 H, J = 8.7 Hz, C$\underline{H}$O), 7.44 (ddd, 1 H, J = 15.4, 11.4, 0.6 Hz), 7.17 (ddd, 1 H, J = 15.4, 11.4, 0.6 Hz), 6.42 (ddt, 1 H, J = 15.4, 11.7, 0.6 Hz), 6.32 (dt, J = 15.4, 0.6 Hz), 3.82 (s, 3 H, OC$\underline{H}_3$); ^{13}C NMR (90 MHz, acetone-d_6) δ 193.97 ($\underline{C}$HO), 166.46 ($\underline{C}$OOCH$_3$), 148.25, 141.77, 138.11, 129.92, 52.09 (O$\underline{C}$H$_3$); MS (EI) calcd for $C_7H_8O_3$ 140.0473, 140.0476 (M).

NAC [1,2-$^{13}C_2$]-7-Hydroxyoct-2-enoate (82d). A similar procedure to that of Kelly *et al.* was used.[115] To a solution of the silyl ether **130d** (290 mg, 0.772 mmol) in dry CH_2Cl_2 (10 mL) was added distilled $BF_3{\cdot}OEt_2$ (1.6 mL) at 0 °C and the resulting mixture was stirred 3 h at 0 °C. The mixture was poured into aqueous 10%

Na_2CO_3 (20 mL) and extracted with CH_2Cl_2 (3 x 20 mL). The organic extracts were dried (Na_2SO_4) and concentrated *in vacuo* to give an oily residue, which was purified by column chromatography (silica, 50% EtOAc in hexanes, then EtOAc) to afford the β-hydroxy thioester **82d** (106 mg, 53%) with recovery of the starting silyl ether **130d** (136 mg, 47%). IR ($CHCl_3$ cast) 3280 (br), 2918 (m), 1658 (s), 1552 (m) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.18 and 6.72 (dm, 1 H, J = 154 Hz, $^{13}C\underline{H}={}^{13}CHCO$), 6.38 and 5.94 (dm, 1 H, J = 161 Hz, $^{13}CH={}^{13}C\underline{H}CO$), 5.90 (br s, 1 H, $N\underline{H}$), 3.80 (dt, 1 H, J = 5.9, 5.4 Hz, $C\underline{H}(OH)$), 3.47 (dd, 2 H, J = 6.4, 5.4 Hz, $C\underline{H}_2NH$), 3.09 (t, 2 H, J = Hz, $S C\underline{H}_2$), 2.24 (m, 2 H, $C\underline{H}_2{}^{13}CH$), 1.97 (s, 3 H, $COC\underline{H}_3$), 1.7 -1.4 (m, 4 H, $CH(OH)C\underline{H}_2C\underline{H}_2$), 1.18 (d, 3 H, J = 6.4 Hz, $C\underline{H}_3CH(OH)$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 190.34 (d, J = 62.0 Hz, $\underline{C}OS$), 170.35 ($\underline{C}OCH_3$), 146.18 (d, J = 70.0 Hz, enriched, $^{13}\underline{C}H={}^{13}CHCO$), 128.53 (d, J = 70.0 Hz, enriched, $^{13}CH={}^{13}\underline{C}HCO$), 67.70 ($\underline{C}H(OH)$), 39.79 ($CO\underline{C}H_3$), 38.55 (d, J = 3.0 Hz, $CH(OH)\underline{C}H_2$), 32.10 (d, J = 41.0 Hz, $\underline{C}H_2{}^{13}CH$), 28.26 ($S\underline{C}H_2$), 24.12 (dd, J = 4.0, 2.0 Hz, $\underline{C}H_2CH_2{}^{13}CH$), 23.64 ($\underline{C}H_3CH(OH)$), 23.18 ($\underline{C}H_2NH$); MS (EI) calcd for $^{13}C_2C_{10}H_{21}NO_3S$ 261.1278, found 261.1218 (M).

For unlabeled material (**82a**): IR ($CHCl_3$ cast) 3238 (br), 2949 (m), 1663 (s), 1655 (shoulder), 1558 (m) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 6.92 (dt, 1 H, J = 15.6, 7.1 Hz, $C\underline{H}=CHCO$), 6.30 (br s, 1 H, $N\underline{H}$), 6.12 (dt, 1 H, J = 15.6, 1.5 Hz, $CH=C\underline{H}CO$), 3.78 (m, 1 H, $C\underline{H}(OH)$), 3.42 (dt, 2 H, J = 6.8, 5.4 Hz, $C\underline{H}_2NH$), 3.07 (t, 2 H, J = 6.8 Hz, $SC\underline{H}_2$), 2.02 (m, $C\underline{H}_2CH=CH$), 1.93 (s, 3 H, $COC\underline{H}_3$), 1.7 -1.4 (m, 4 H, $CH(OH)C\underline{H}_2C\underline{H}_2$), 1.15 (d, 3 H, J = 6.3 Hz, $C\underline{H}_3CH(OH)$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 190.22 ($\underline{C}OS$), 170.39 ($\underline{C}OCH_3$), 146.13 ($\underline{C}H=CHCO$), 128.41 ($CH=\underline{C}HCO$), 67.46 ($\underline{C}H(OH)$), 39.61 ($CO\underline{C}H_3$), 38.46 ($CH(OH)\underline{C}H_2$), 32.03 ($\underline{C}H_2CH=CH$), 28.14 ($S\underline{C}H_2$), 24.02 ($\underline{C}H_2CH_2CH=CH$), 23.51 ($\underline{C}H_3CH(OH)$), 23.07 ($\underline{C}H_2NH$); MS (CI, NH_3) 260 (MH^+, 100); Anal. Calcd for $C_{12}H_{21}NO_3S$: C, 55.77; H, 8.16; N, 5.40. Found: C, 54.71; H, 8.07; N, 5.02.

NAC (*S*)-[6,7-$^{13}C_2$,7-*hydroxy*-^{18}O]-7-Hydroxyoct-2-enoate (82f). The method for the conversion of **57d** to **35d** was used. Thus, **130f** (32.9 mg, 0.0875 mmol) gave **82f** (23.1 mg, 100%). IR (CH_2Cl_2 cast) 3378 (br), 2930 (m), 1658 (s), 1634 (m), 1558 (m) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.93 (dt, 1 H, *J* = 15.6, 7.0 Hz, $C\underline{H}=CHCO$), 6.15 (dt, 1 H, *J* = 15.6, 1.5 Hz, $CH=C\underline{H}CO$), 5.87 (br s, 1 H, $N\underline{H}$), 3.98 and 3.63 (dm, 1 H, *J* = 141 Hz, $^{13}C\underline{H}(^{18/16}OH)$), 3.46 (q, 2 H, *J* = 6.2 Hz, $C\underline{H_2}NH$), 3.09 (t, 2 H, *J* = 6.3 Hz, $SC\underline{H_2}$), 2.23 (m, 2 H, $C\underline{H_2}CH=CH$), 1.97 (s, 3 H, $COC\underline{H_3}$), 1.7-1.4 (m, 4 H, $^{13}C\underline{H_2}C\underline{H_2}$), 1.21 (dt, 3 H, *J* = 6.0, 4.4 Hz, $C\underline{H_3}{}^{13}CH(^{18/16}OH)$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.40 ($\underline{C}OS$), 170.25 ($\underline{C}OCH_3$), 146.18 (d, *J* = 2.6 Hz, $\underline{C}H=CHCO$), 128.55 ($CH=\underline{C}HCO$), 67.76 (dd, *J* = 38.3, 2.0 Hz, enriched, $^{13}\underline{C}H(^{18/16}OH)$), 39.82 ($CO\underline{C}H_3$), 38.52 (d, *J* = 38.4 Hz, enriched, $^{13}\underline{C}H_2$), 32.20 (d, *J* = 4.2 Hz, $\underline{C}H_2CH=CH$), 28.29 ($S\underline{C}H_2$), 24.12 (t, *J* = 17.3 Hz, $^{13}CH_2\underline{C}H_2$), 23.67 (t, *J* = 19.2 Hz, $\underline{C}H_3{}^{13}CH(^{18/16}OH)$), 23.23 ($\underline{C}H_2NH$); MS (CI, NH_3) 262 (MH^+(^{16}O), 41.4), 264 (MH^+ (^{18}O), 77.8) .

Isolation of Hypoglycin (83) from Ackee Fruit Seeds. The method reported by Billington and coworkers was followed.[188a] The ackee fruit seeds (1 kg) were ground to a fine power with a grinder, extracted with 80% EtOH (2 L), and then filtered. The brown syrup (ca. 50 g) after the concentration of the filtrate was taken up in 0.1 N HCl to 150 mL and centrifuged, the resulting supernatant was chromatographed on AG 50W-X8 (H^+ form, 500 g, 4.5 x 35 cm) with 0.1N HCl (800 mL) followed 1 N pyridine. The fractions (100 mL/fraction) were followed by TLC (silica, propanol/H_2O = 70/30, R_f 0.61 for **83**, ninhydrin spray detection). Crude **83** (from fractions 19 to 23) (2.30 g) was obtained and further purified on an ion exchange column (AG 1-X8, ACO^- form, 4.5 x 30 cm) by eluting successively with H_2O (250 mL), 0.1 N AcOH (250 mL), 0.5 N AcOH (500 mL), 1 N AcOH (500 mL) and 3 N AcOH. Hypoglycin (**83**) (1.61 g) was obtained from concentration of fractions 32 to 60 (25 mL/fraction), which was further purified by recrystallization from 70% EtOH/H_2O. Mp 240 °C (dec.); IR (KBr) 3440 (br m), 3424 (br m), 1583 (br s), 1516 (m) cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 5.36 (m, 2 H), 3.70-3.46 (m, 1 H), 1.75 (m, 2 H), 1.25 (m, 1 H), 0.75 (m, 1 H); ^{13}C NMR (50 MHz, D_2O) δ 176.00 (reference), 136.23, 105.59, 56.58, 35.40, 12.34, 10.53; MS (EI) calcd for $C_7H_{10}NO_2$ 140.0711, found 140.0709 (M-H).

3-Octylthiopropanoic Acid (86a). A procedure similar to that of Spydevold and Bremer was used.[187] 1-Bromooctane **115a** (5.40 g, 80.0 mmol) was added to a solution of 3-mercaptopropanoic acid **116** (16.9 g, 160 mmol) and KOH (17.2 g, 307 mmol) in methanol (200 mL) over 30 min. The resulting mixture was stirred overnight at room temperature and filtered to remove the white precipitate (KBr). The filtrate was concentrated *in vacuo* and the resulting white solid was redissolved in H_2O (100 mL). This was acidified by 2N HCl, the white crystals were collected and dried to afford the known[187] acid **86a** (17.1 g, 98%). Mp 41.0-42.0 °C; IR (KBr disk) 3600-2400 (br),2957 (m), 2918 (s), 2850 (s), 1685 (s) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 2.75 (m, 2 H), 2.67 (m, 2 H), 2.54 (t, 2 H, J = 7.3 Hz, $C\underline{H}_2COOH$), 1.58 (m, 2 H), 1.40-1.17 (m, 10 H), 0.87 (m, 3 H, $C\underline{H}_3$); ^{13}C NMR (50 MHz, DMSO-d_6) δ 172.95 ($\underline{C}OOH$), 34.61, 31.30, 31.12, 29.13, 28.66 (2 x C), 28.29, 26.45, 22.12, 13.92 ($\underline{C}H_3$); MS (EI) calcd for $C_{11}H_{22}O_2S$ 218.1340, found 218.1339 (M).

3-Tetradecylthiopropanoic Acid (86b). The method for making of **86a** was followed. Thus, 1-tetradecylbromide **115b** (27.9 g, 100 mmol) and 3-mercaptopropanoic acid **116** (27.6 g, 260 mmol) gave the known[187] acid **86b** (28.6 g, 95%). Mp 69.0-70.0 °C; IR (KBr disk) 3600-2500 (br),2955 (m), 2918 (s), 2847 (m), 1685 (s) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 2.75 (m, 2 H), 2.67 (m, 2 H), 2.52 (t, 2 H, J = 7.3 Hz, $C\underline{H}_2COOH$), 1.58 (m, 2 H), 1.48-1.20 (m, 22 H), 0.85 (m, 3 H, $C\underline{H}_3$); ^{13}C NMR (50

MHz, $CDCl_3$) δ 177.85 (COOH), 34.67, 32.23, 31.94, 29.66 (very strong), 29.55 (2 x C), 29.36, 29.22, 28.69, 26.61, 22.71, 14.11 (CH_3); MS (EI) calcd for $C_{17}H_{34}O_2S$ 302.2279, found 302.2290 (M).

Ethyl 3-Hydroxypent-4-ynoate (102). A procedure similar to the method of Takahata was used.[184] A mixture of tetrabutylammonium fluoride (1.47 g, 7.95 mmol) and the silyl compound **110** (1.13 g, 5.30 mmol) in THF (15 mL) was stirred at room temperature for 1 h and diluted with ether (20 mL). This was washed with brine (2 x 20 mL), dried ($MgSO_4$), and concentrated to afford a brown oil, which was distilled to give **102** (530 mg, 77%). Bp 55 °C (0.01 mm Hg); IR (neat) 3600-3200 (br), 2964 (m), 2937 (m), 2876 (m), 1732 (s), 1467 (m), 1448 (m), 1398 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.61 (dt, 1 H, *J* = 7.0, 2.5 Hz, CH(OH)), 4.09 (q, 2 H, *J* = 7.2 Hz, OCH_2), 2.60 (d, 2 H, *J* = 7.0 Hz, CH_2COOEt), 1.12 (t, 3 H, *J* = 7.2 Hz, CH_2CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.46 (COOEt), 90.35, 72.87, 60.57 (CH(OH)), 57.94 (OCH_2), 41.85 (CH_2COOEt), 13.67 (CH_3); MS (CI, NH_3) 143 (MH^+, 24.1), 160 (MNH_4^+, 100).

***N*-(4-Pentynoyl) Glycine (103).** An aqueous solution of 1 N NaOH (1.3 mL) was added dropwise to the benzyl ester **107** (317 mg, 1.29 mmol) in THF-H_2O (9.1 mL/2.3 mL) over 5 min. The reaction mixture was stirred at room temperature for 30 min, and was then acidified with 0.1 N HCl (20 mL). The aqueous layer was extracted with $CHCl_3$ (3 x 50 mL). The combined organic phases were dried over Na_2SO_4 and concentrated to give a yellowish solid (180 mg). The solid residue was chromatographed on silica gel (EtOAc, then 1% MeOH in EtOAc) to give **103** (31.0 mg, 15%). Mp 110-112 °C; IR (acetone cast) 3400-3200 (br), 3281 (m), 1728 (s), 1642 (s), 1552 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.45 (br s, 1 H, NH), 3.94 (d, 2 H, *J* = 5.7 Hz, $NHCH_2$), 2.60-2.40 (m, 4 H, CH_2CH_2CO), 2.34 (t, 1 H, *J* = 2.0 Hz, acetylenic-H); ^{13}C NMR (90

MHz, acetone-d_6) δ 171.76, 171.29, 84.00, 70.05, 35.33, 35.29, 15.00; MS (EI) calcd for $C_7H_9NO_3$ 155.0582, found 155.0576 (M).

Benzyl *N*-(4-Pentynoyl) Glycinate (107). The procedure of Yokoyama and coworkers was used.[182] To a cold (0 °C) solution of 4-pentynoic acid **101** (300 mg, 3.06 mmol) and benzyl glycinate (*p*-TsOH salt) (105 mg, 3.10 mmol) in DMF (10 mL) was added diphenylphosphoryl azide (668 μL, 853 mg, 3.10 mmol). The reaction mixture was then treated with Et_3N (846 μL) in DMF (10 mL) and stirred for 5 h at 0 °C. The mixture was diluted with benzene (100 mL) and EtOAc (200 mL), and was washed sequentially with 5% HCl (2 x 100 mL), H_2O (100 mL), 10% Na_2CO_3 (2 x 50 mL), and H_2O (100 mL). The organic phase was dried (Na_2SO_4) and concentrated to give a colorless liquid (1.20 g), which was purified chromatographically (silica, 40% EtOAc in hexanes, R_f 0.24) to afford **107** (716 mg, 94%). Mp 60.2-62.0 °C; IR ($CHCl_3$, cast) 3314 (m), 1736 (s), 1655 (s), 1545 (m), 1416 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.38 (m, 5 H, Ar$\underline{H}$), 6.18 (br s, 1 H, N$\underline{H}$), 5.22 (s, 2 H, OC$\underline{H}_2$), 4.13 (d, 2 H, *J* = 5.0 Hz, NHC$\underline{H}_2$), 2.55 (m, 4 H, C$\underline{H}_2$C$\underline{H}_2$CO), 2.04 (t, 1 H, *J* = 2.0 Hz, acetylenic-$\underline{H}$); ^{13}C NMR (90 MHz, $CDCl_3$) δ 171.64, 170.34, 137.11, 129.24, 128.88, 128.85, 70.04, 66.92, 41.72, 35.27, 14.96; MS (EI) calcd for $C_{14}H_{15}NO_3$ 245.1052, found 245.1049 (M).

3-Trimethylsilyl-2-propynal (109). The method of Kruithof was followed.[183] Trimethylsilylacetylene **108** (5.00 g, 50.9 mmol) in THF (40 mL) was added to ethylmagnesium bromide [made by adding of ethyl bromide (8.39 g, 77.0 mmol) to magnesium turnings (1.68 g, 70.0 mmol) in THF (20 mL)] over 20 min at 0 °C. The mixture was stirred at room temperature for 1 h, transferred into a dropping funnel, and added to a solution of DMF (14.0 g, 191 mmol) in ether (25 mL) over 45 min at -25 °C. The mixture was warmed to room temperature and stirred for 1 h, poured into ice cold 5%

H_2SO_4 (100 mL), and the aqueous solution was extracted with ether (3 x 100 mL). A trace of hydroquinone was added to the organic extracts, and this was concentrated to afford a brown oil, which was distilled at 45-46 °C (water pump) to give the known[183] the aldehyde **109** (4.82 g, 80%). IR (neat) 1682 (s), 1668 (s), 1254 (m), 1000 (s) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 9.11 (s, 1 H, $C\underline{H}O$), 0.18 (s, 9 H, $(C\underline{H}_3)_3Si$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 176.38 ($\underline{C}HO$), 102.49, 102.08, -1.14 ($(\underline{C}H_3)_3Si$); MS (EI) calcd for $C_6H_{10}OSi$ 126.0501, found 126.0446 (M).

Ethyl 5-Trimethylsilyl-3-hydroxypent-4-ynoate (110). A procedure similar to the method of Martin was used.[110] EtOAc (734 mg, 8.33 mmol) was added to LiHMDS [freshly made by adding of 1.00 M *n*-BuLi (8.33 mL, 8.33 mmol) in hexanes to HMDS (1.34 g, 8.33 mmol) in THF (5 mL) at 0 °C] over 15 min at -78 °C. The enolate solution was stirred for 30 min before addition of the aldehyde **109** (1.05 g, 8.33 mmol) at -78 °C. The reaction was continued for 30 min at -78 °C and then allowed to warm to room temperature for a further 30 min. The mixture was poured into saturated ammonium chloride (100 mL), and the resulting aqueous solution was extracted with ether EtOAc (3 x 50 mL). The combined organic phases were dried over $MgSO_4$ and concentrated to give **110** (1.37 g, 77%). IR (neat) 3600-3200 (br), 2961 (m), 2937 (m), 2876 (m), 1739 (s), 1466 (m), 1448 (m), 1397 (m), 1374 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.70 (dt, 1 H, J = 6.0, 2.1 Hz, $C\underline{H}(OSi)$), 4.09 (q, 2 H, J = 7.1 Hz, $OC\underline{H}_2$), 2.66 (d, 2 H, J = 6.0 Hz, $C\underline{H}_2COOEt$), 0.90 (t, 3 H, J = 7.1 Hz, $CH_2C\underline{H}_3$), 0.06 (s, 9 H, $(C\underline{H}_3)_3Si$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.16 ($\underline{C}OOEt$), 106.47, 104.48, 60.82 ($\underline{C}H(OSi)$), 59.03 ($O\underline{C}H_2$), 42.15 ($\underline{C}H_2COOEt$), 14.09 ($\underline{C}H_3$), -0.34 ($(\underline{C}H_3)_3Si$); MS (CI, NH_3) 215 (MH^+, 91.5).

5-Hexynoic Acid (114). A modification of the method of Green *et al.* was used.[185] To a cold (0 °C) solution of sodium persulfate (5.30 g, 22.3 mmol) in aqueous 1N NaOH (100 mL) was sequentially added ruthenium trichloride trihydrate ($RuCl_3{\cdot}3H_2O$)

(130 mg), and 5-hexynol (**113**) (1.04 g, 10.6 mmol) in CCl_4 (20 mL). The reaction was continued for 3 h at 0 °C and the mixture was then extracted with ether (100 mL). After removal of ether, the organic phase gave the starting alcohol (12%). The aqueous phase was acidified to pH 3 with 6N HCl and extracted with ether (3 x 50 mL). The extracts were combined, dried (Na_2SO_4), and concentrated to afford **114** (885 mg, 76%). IR ($CHCl_3$ cast) 3400-3200 (br), 3285 (m), 1709 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.52 (t, 2 H, *J* = 7.3 Hz, $C\underline{H}_2CO$), 2.29 (dt, 2 H, *J* = 6.9, 2.6 Hz, $C\underline{H}_2CH_2CH_2CO$), 1.99 (t, 1 H, *J* = 2.6 Hz, acetylenic-$\underline{H}$), 1.72 (tt, 2 H, *J* = 7.2, 6.9 Hz, $C\underline{H}_2CH_2CO$); ^{13}C NMR (90 MHz, $CDCl_3$) δ 179.64, 82.99, 69.30, 32.58, 23.24, 17.71; MS (EI) calcd for $C_6H_8O_2$ 112.0524, found 112.0516 (M).

2-Tetrahydropyranyl 3-Butenyl Ether (117). The procedure of Kohn *et al.* was followed.[189b] A mixture of 3-butenol (7.21 g, 100 mmol), dihydropyran (10.1 g, 120 mmol), and conc. HCl (50 μL) was stirred for 3 h at room temperature. The mixture was distilled to give **117** (14.3 g, 92%). Bp 63-65 °C (4 mm Hg); IR ($CHCl_3$ cast) 2942 (s), 2870 (m), 1123 (m), 1077 (m), 1035 (s) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 5.92-5.70 (m, 1 H, $CH_2{=}C\underline{H}$), 5.10-4.94 (m, 2 H, $CH{=}C\underline{H}_2$), 4.55 (m, 1 H, $CH_2OC\underline{H}O$), 3.90-3.66 (m, 2 H, $C\underline{H}_2O$), 3.50-3.30 (m, 2 H, $C\underline{H}_2O$), 2.26 (m, 2 H), 1.90-1.30 (m, 6 H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 135.18, 116.11, 98.60, 66.65, 62.10, 34.08, 30.58, 25.39, 19.44; MS (EI) calcd for $C_9H_{16}O_2$ 156.1150, found 156.1115 (M).

2-[2-(1-Chloro-1-methylcycloprop-2-yl)ethoxy]tetrahydro-2*H*-pyran (118). The procedure of Baldwin *et al.* was used.[189a] To a mixture of **117** (13.5 g, 86.4 mmol) and 1, 1-dichloroethane (10.3 g, 103 mmol) in ether (20 mL) was added n-BuLi (1.5 M, 63.0 mL, 94.0 mmol) in hexanes over 1.5 h at -40 °C. The reaction mixture was stirred at room temperature for 12 h. H_2O (20 mL) was added to the reaction mixture, the organic phase was separated, dried (Na_2SO_4), and concentrated to give a yellow liquid (17.0 g). Distillation of the residue at reduced pressure afforded the known[189a] compound **118** (6.28 g, 33%) with recovery of the starting alkene (8.68 g, 64%). Bp 93 °C (1.5 mm Hg), lit[189a] bp 50-55 °C (0,05 mm Hg); IR ($CHCl_3$ cast) 2942 (s), 2890 (m), 1201 (m), 1184 (m), 1036 (s) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 4.65 (m, 1 H, $CH_2OC\underline{H}O$), 3.98-3.80 (m, 2 H, $C\underline{H}_2O$), 3.62-3.42 (m, 2 H, $C\underline{H}_2O$), 2.00-1.50 (m, 13 H), 1.00-0.87 (m, 1 H); ^{13}C NMR (90 MHz, $CDCl_3$) δ ; MS (EI) calcd for $C_{11}H_{19}ClO$ 218.1074, found 218.1061 (M).

2-[2-(Methylenecycloprop-2-yl)ethoxy]tetrahydro-2*H*-pyran (119). The procedure of Baldwin *et al.* was used.[189a] A mixture of potassium *tert*-butoxide (3.30 g, 29.4 mmol) and **118** (6.00 g, 29.6 mmol) in dry DMSO (10 mL) was heated for 6 h at 70 °C and 8 h at room temperature. The mixture was poured onto crushed ice (ca. 200 mL) and extracted with ether (200 mL). The ether phase was washed with brine (3 x 100 mL), dried (Na_2SO_4), and concentrated to give a brown liquid (4.70 g), which was flash chromatographed on silica gel (10% EtOAc in hexanes, R_f 0.33) to afford the known[189a] compound **119** (4.28 g, 86%). IR ($CHCl_3$ cast) 2930 (m), 2863 (m), 2360 (m), 2328 (m), 1122 (m), 1036 (s) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 5.48-5.34 (m, 2 H, $C\underline{H}_2{=}C$), 4.65 (m, 1 H, $CH_2OC\underline{H}O$), 4.00-3.78 (m, 2 H, $C\underline{H}_2O$), 3.60-3.43 (m, 2 H, $C\underline{H}_2O$), 1.92-1.45 (m, 9 H), 1.34-1.20 (m, 1 H), 0.97-0.66 (m, 1 H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 136.26, 102.83, 98.72 and 98.67 ($CH_2O\underline{C}HO$), 67.16 and 67.13 ($\underline{C}H_2O$), 62.14 and 62.05 ($\underline{C}H_2O$), 33.29 and 33.21, 30.67, 25.47, 19.52 and 19.45, 12.90, 9.29 and 9.21; MS (EI) calcd for $C_{11}H_{18}O_2$ 182.1307, found 182.1304 (M).

2-(Methylenecycloprop-2-yl)ethanol (120). The procedure of Baldwin *et al.* was used.[189a] The THP ether **119** (4.01 g, 22.0 mmol) was dissolved in MeOH (100 mL), and *p*-toluenesulfonic acid monohydrate (1.20 g, 6.31 mmo) was added. The mixture was stirred for 21 h at room temperature, and then K_2CO_3 (1.20 g) was added.

Solvent was removed *in vacuo*, the resulting residue was dissolved in H_2O (200 mL) and extracted with $CHCl_3$ (3 x 50 mL). The dried (Na_2SO_4) organic phases were concentrated to give the known[189a] alcohol **120** (2.16 g, 99%). IR ($CHCl_3$ cast) 3270 (br), 2931 (m), 2877 (m), 1442 (m), 1380 (m), 1030 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 5.35 (m, 2 H, $C\underline{H}_2$=C), 3.75 (t, 2 H, J = 6.0 Hz, $C\underline{H}_2O$), 2.13(br s, 1 H, $O\underline{H}$), 1.80-1.30 (m, 3 H), 1.28-1.13 (m, 1 H), 0.88-0.60 (m, 1 H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 135.85, 103.00, 62.56 ($\underline{C}H_2OH$), 35.89, 12.41, 9.09; MS (EI) calcd for $C_6H_{10}O$ 98.0732, found 98.0727 (M).

Methylenecyclopropaneacetic Acid (121). The procedure of Baldwin *et al.* was used.[189a] To the alcohol **120** (2.03 g, 20.6 mmol) in acetone was added Jones' reagent (16 mL) over 30 min at -20 °C. The mixture was then kept at -5 °C for 4 h. 2-Propanol (2 mL) and conc. HCl (2 mL) were added to the reaction mixture. The supernatant was decanted into a separatory funnel and extracted with ether (3 x 100 mL). The combined organic phases were extracted with aqueous 10% NaOH (200 mL). The basic solution was acidified to pH 1 with conc. HCl and extracted with ether (3 x 100 mL). The dried ether phases were concentrated to give the known[189a] acid **121** (1.82 g, 78%). IR ($CHCl_3$ cast) 3400-2400 (br), 1710 (s), 1420 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.58 (m, 1 H, $C\underline{H}_2$=C), 5.48 (m, 1 H, $C\underline{H}_2$=C), 2.41 (br d, 2 H, J = 7.2 Hz, $C\underline{H}_2$), 1.73 (m, 1 H), 1.40 (ddt, 1 H, J = 9.0, 2.3, 1.8 Hz), 0.91 (ddd, 1 H, J = 9.0, 5.0, 2.4 Hz); ^{13}C NMR (90 MHz, $CDCl_3$) δ 179.01 ($\underline{C}OOH$), 133.99, 104.33, 37.62, 10.20, 9.40; MS (EI) calcd for $C_6H_{10}O_2$ 112.0524, found 112.0517 (M).

NAC Methylenecyclopropaneacetate (122). The method for conversion of **56d** to **57d** was used. Thus, the acid **121** (800 mg, 7.10 mmol) gave **122** (670 mg, 44%) as a gum after column chromatography (silica, EtOAc, R_f 0.33). IR ($CHCl_3$ cast) 3288 (m), 3077 (w), 2930 (m), 1687 (s), 1654 (s), 1552 (m), 1288 (m), 1030 (m) cm^{-1};

^{1}H NMR (300 MHz, $\mathrm{CDCl_3}$) δ 5.85 (br s, 1 H, $\mathrm{N\underline{H}}$), 5.85 (m, 1 H, $\mathrm{C\underline{H}_2{=}C}$), 5.45 (m, 1 H, $\mathrm{C\underline{H}_2{=}C}$), 3.46 (dt, 2 H, *J* = 6.4, 5.7 Hz, $\mathrm{C\underline{H}_2NH}$), 3.03 (t, 2 H, *J* = 6.4 Hz, $\mathrm{SC\underline{H}_2}$), 2.58 (br d, 2 H, *J* = 7.2 Hz, $\mathrm{C\underline{H}_2}$), 1.73 (m, 1 H), 1.41 (ddt, 1 H, *J* = 9.0, 2.3, 1.8 Hz), 0.93 (ddd, 1 H, *J* = 9.0, 5.0, 2.4 Hz); ^{13}C NMR (90 MHz, $\mathrm{CDCl_3}$) δ 198.98 and 198.94 ($\mathrm{\underline{C}OS}$), 170.24 and 170.22 ($\mathrm{\underline{C}OCH_3}$), 133.66, 104.59, 47.06 and 47.05, 39.61 and 39.55, 28.43, 23.15, 11.37, 9.51; MS (CI, $\mathrm{NH_3}$) 214 ($\mathrm{MH^+}$, 100).

Ethyl *(S)*-[1-^{13}C]-5-(*tert*-Butyldimethylsiloxy)hexanoate (123c). The procedure of Ernst *et al.* was used.[191] The α,β-unsaturated ester **69c** (1.82 g, 6.66 mmol) was dissolved in EtOAc (50 mL), and 5% palladium carbon (180 mg) was added. Hydrogenation was continued for 3 h at room temperature and then the mixture was filtered. Concentration of the filtrate gave **123c** (1.72 g, 95%). IR ($\mathrm{CH_2Cl_2}$ cast) 2957 (m), 2930 (m), 2857 (m), 1697 (s) cm^{-1}; ^{1}H NMR (360 MHz, $\mathrm{CDCl_3}$) δ 4.08 (dq, 2 H, *J* = 7.0, 3.1 Hz, $\mathrm{OC\underline{H}_2}$), 3.76 (m, 1 H, $\mathrm{C\underline{H}(OSi)}$), 2.23 (m, 2 H, $\mathrm{C\underline{H}_2COO}$), 1.6-1.3 (m, 4 H, $\mathrm{CH(OSi)C\underline{H}_2C\underline{H}_2}$), 1.19 (t, 3 H, *J* = 7.0 Hz, $\mathrm{CH_2C\underline{H}_3}$), 1.12 (d, 3 H, *J* = 6.1 Hz, $\mathrm{C\underline{H}_3CH(OSi)}$), 0.87 (s, 9 H, $\mathrm{(C\underline{H}_3)_3C}$), 0.06 (s, 6 H, $\mathrm{(C\underline{H}_3)_2Si}$); ^{13}C NMR (100 MHz, $\mathrm{CDCl_3}$) δ 173.68 (enriched, $\mathrm{\underline{C}OO}$), 68.18($\mathrm{\underline{C}H(OSi)}$), 60.12 ($\mathrm{O\underline{C}H_2}$), 38.97 ($\mathrm{CH(OSi)\underline{C}H_2}$), 34.33 (d, *J* = 57.4 Hz, $\mathrm{\underline{C}H_2{}^{13}COO}$), 25.87 ($\mathrm{(\underline{C}H_3)_3C}$), 23.70 ($\mathrm{\underline{C}H_3CH(OSi)}$), 21.29 ($\mathrm{\underline{C}H_2CH_2{}^{13}COO}$), 18.11 ($\mathrm{(CH_3)_3\underline{C}}$), -4.41 and -4.77 ($\mathrm{(\underline{C}H_3)_2Si}$); MS (CI, $\mathrm{NH_3}$) 276 ($\mathrm{MH^+}$, 51).

For unlabeled racemic material (**123a**): IR ($\mathrm{CH_2Cl_2}$ cast) 2958 (m), 2929 (m), 2858 (m), 1740 (s) cm^{-1}; ^{1}H NMR (360 MHz, $\mathrm{CDCl_3}$) δ 4.12 (q, 2 H, *J* = 7.1 Hz, $\mathrm{OC\underline{H}_2}$), 3.78 (ddq, 1 H, *J* = 6.4, 5.9, 5.4 Hz, $\mathrm{C\underline{H}(OSi)}$), 2.29 (t, 2 H, *J* = 7.6 Hz, $\mathrm{C\underline{H}_2COO}$), 1.6-1.3 (m, 4 H, $\mathrm{CH(OSi)C\underline{H}_2C\underline{H}_2}$), 1.24 (t, 3 H, *J* = 7.1 Hz, $\mathrm{CH_2C\underline{H}_3}$), 1.12 (ddd, 3 H, *J* = 5.9, 1.5, 0.98 Hz, $\mathrm{C\underline{H}_3CH(OSi)}$), 0.87 (s, 9 H, $\mathrm{(C\underline{H}_3)_3C}$), 0.06 (s, 6 H, $\mathrm{(C\underline{H}_3)_2Si}$); ^{13}C NMR (100 MHz, $\mathrm{CDCl_3}$) δ 173.69 ($\mathrm{\underline{C}OO}$), 68.18($\mathrm{\underline{C}H(OSi)}$), 60.15 ($\mathrm{O\underline{C}H_2}$), 38.99 ($\mathrm{CH(OSi)\underline{C}H_2}$), 34.38 ($\mathrm{\underline{C}H_2{}^{13}COO}$), 25.87 ($\mathrm{(\underline{C}H_3)_3C}$), 23.71

($\underline{C}H_3CH(OSi)$), 21.28 ($\underline{C}H_2CH_2COO$), 18.11 ($(CH_3)_3\underline{C}$), 14.23 ($CH_2\underline{C}H_3$), -4.41 and -4.77 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) 275 (MH^+, 75); Anal. Calcd for $C_{14}H_{30}O_3Si$: C, 61.26; H, 11.02. Found: C, 61.06; H, 11.10.

Ethyl *(S)*-[4,5-$^{13}C_2$]-5-(*tert*-Butyldimethylsiloxy)hexanoate (123d). The method for the conversion of **69c** to **123c** was used. Thus, the α,β-unsaturated ester **69d** (1.05 g, 3.83 mmol) gave **123d** (1.06 g, 100%). IR (CH_2Cl_2 cast) 2958 (m), 2929 (m), 2858 (m), 1740 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.14 (q, 2 H, J = 7.1 Hz, $OC\underline{H}_2$), 3.97 and 3.63 (dm, 1 H, J = 140 Hz, $^{13}C\underline{H}(OSi)$), 2.31 (m, 2 H, $C\underline{H}_2COO$), 1.7-1.5 (m, 4 H, $^{13}C\underline{H}_2C\underline{H}_2$), 1.27 (t, 3 H, J = 7.1 Hz, $CH_2C\underline{H}_3$), 1.14 (dt, 3 H, J = 6.0, 4.4 Hz, $C\underline{H}_3{}^{13}CH(OSi)$), 0.87 (s, 9 H, $(C\underline{H}_3)_3C$), 0.07 (s, 6 H, $(C\underline{H}_3)_2Si$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.74 (d, J = 2.6 Hz, $\underline{C}OO$), 68.25 (d, J = 39.2 Hz, enriched, $^{13}\underline{C}H(OSi)$), 60.17 ($O\underline{C}H_2$), 38.99 (d, J = 39.7 Hz, enriched, $^{13}\underline{C}H_2$), 34.38 (d, J = 4.0 Hz, $\underline{C}H_2COO$), 25.89 ($(\underline{C}H_3)_3C$), 23.72 (t, J = 19.6 Hz, $\underline{C}H_3{}^{13}CH(OSi)$), 21.30 (t, J = 17.5 Hz, $^{13}CH_2\underline{C}H_2$), 18.12 ($(CH_3)_3\underline{C}$), -4.40 and -4.76 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) 277 (MH^+, 72).

***(S)*-[1-^{13}C]-5-(*tert*-Butyldimethylsiloxy)hexanol (124c).** A similar method to that used by Nicolaou *et al.* was employed.[41b] To a solution of the ester **123c** (1.69 g, 6.14 mmol) in CH_2Cl_2 (15 mL) was added DIBAL (2.62 g, 18.4 mmol) in CH_2Cl_2 (10 mL) over 10 min at -78 °C. The reaction mixture was stirred for 2 h at -78 °C, and 30 min at -30 °C. Then MeOH (2 mL) was added to quench the excess of DIBAL. The mixture was diluted with ether (300 mL) and the ether phase was washed with saturated potassium-sodium tartrate (4 x 100 mL), and brine (3 x 100 mL), dried over Na_2SO_4, and concentrated to give a liquid residue (1.40 g), which was purified by column chromatography (silica, 4.5 x 10 cm, 20% ether in pentane, R_f 0.16) to afford **124c** (1.14 g, 80%). $[\alpha]_D$ +11.1° (c 0.49 $CHCl_3$); IR (CH_2Cl_2 cast) 3335 (br), 2951(m), 2930 (m), 2858 (m), 1255 (m) cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 3.80 (m, 1 H, $C\underline{H}(OSi)$), 3.45 (m, 2 H, $^{13}C\underline{H}_2OH$), 1.64-1.24 (m, 6 H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 1.12 (d, 3 H, J = 6.4 Hz, $C\underline{H}_3CH(OSi)$), 0.87 (s, 9 H, $(C\underline{H}_3)_3C$), 0.07 (s, 6 H, $(C\underline{H}_3)_2Si$); ^{13}C NMR (90 MHz, $CDCl_3$) δ 68.49 ($\underline{C}H(OSi)$), 62.98 (enriched, $^{13}\underline{C}H_2OH$), 39.47 ($CH(OH)\underline{C}H_2$), 32.79 (d, J = 36.6 Hz, $\underline{C}H_2{}^{13}CH_2$), 25.90 ($(\underline{C}H_3)_3C$), 23.75 ($\underline{C}H_3CH(OSi)$), 21.85 ($\underline{C}H_2CH_2{}^{13}CH_2$), 18.15 ($(CH_3)_3\underline{C}$), -4.39 and -4.64 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) 234 (MH^+, 100).

For unlabeled racemic material (**124a**): IR (CH_2Cl_2 cast) 3380 (br), 2958 (m), 2930 (m), 2858 (m), 1255 (m) cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 3.81 (m, 1 H, $C\underline{H}(OSi)$), 3.66 (dt, 2 H, J = 6.8, 5.4 Hz, $C\underline{H}_2OH$), 1.6-1.3 (m, 6 H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 1.13 (d, 3 H, J = 5.9 Hz, $C\underline{H}_3CH(OSi)$), 0.87 (s, 9 H, $(C\underline{H}_3)_3C$), 0.07 (s, 6 H, $(C\underline{H}_3)_2Si$); ^{13}C NMR (90 MHz, $CDCl_3$) δ 68.49 ($\underline{C}H(OSi)$), 62.63 ($\underline{C}H_2OH$), 39.32 ($CH(OH)\underline{C}H_2$), 32.68 ($\underline{C}H_2CH_2OH$), 25.83 ($(\underline{C}H_3)_3C$), 23.68 ($\underline{C}H_3CH(OSi)$), 21.82 ($\underline{C}H_2CH_2CH_2OH$), 18.06 ($(CH_3)_3\underline{C}$), -4.49 and -4.79 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) 233 (MH^+, 7), 257 (MNH_4^+, 13); Anal. Calcd for $C_{12}H_{28}O_2Si$: C, 61.01; H, 12.14. Found: C, 60.96; H, 12.23.

***(S)*-[4,5-$^{13}C_2$]-5-(*tert*-Butyldimethylsiloxy)hexanol (124d).** The method for the conversion of **123c** to **124c** was used. Thus, **123d** (1.05 g, 3.80 mmol) gave the aldehyde **124d** (501 mg, 57%) along with the recovery of **123d** (214 mg, 23%). IR (CH_2Cl_2 cast) 3381 (br), 2956 (m), 2930 (m), 2857 (m), 1255 (m) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.96 and 3.61 (dm, 1 H, J = 140 Hz, $^{13}C\underline{H}(OSi)$), 3.64 (t, 2 H, J = 6.6 Hz, $C\underline{H}_2OH$), 1.65-1.3 (m, 6 H, $^{13}C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 1.12 (dt, 3 H, J = 6.0, 4.3 Hz, $C\underline{H}_3{}^{13}CH(OSi)$), 0.87 (s, 9 H, $(C\underline{H}_3)_3C$), 0.07 (s, 6 H, $(C\underline{H}_3)_2Si$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 68.49 (d, J = 39.5 Hz, enriched, $^{13}\underline{C}H(OSi)$), 62.99 ($\underline{C}H_2OH$), 39.39 (d, J = 39.4 Hz, enriched, $^{13}\underline{C}H_2$), 32.82 (d, J = 4.1 Hz, $\underline{C}H_2CH_2OH$), 25.90 ($(\underline{C}H_3)_3C$), 23.76

(t, *J* = 19.6 Hz, $\underline{C}H_3{}^{13}CH(OSi)$), 21.85 (t, *J* = 17.5 Hz, $^{13}CH_2\underline{C}H_2$), 18.14 ($(CH_3)_3\underline{C}$), -4.38 and -4.72 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) 235 (MH^+, 100).

***(S)*-[1-^{13}C]-5-(*tert*-Butyldimethylsiloxy)hexanal (125c).** The method for conversion of **52c** to **53c** was used. Thus, **124c** (1.12 g, 4.80 mmol) gave **125c** (870 mg, 78%), after column chromatography (silica, 10% ether in pentane, R_f 0.50). IR (CH_2Cl_2 cast) 2956 (m), 2929 (m), 2857 (m), 1688 (s), 1255 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.97 and 9.54 (dt, 1 H, *J* = 170, 1.7 Hz, $^{13}C\underline{H}O$), 3.81(m, 1 H, $C\underline{H}(OSi)$), 2.43 (m, 2 H, $C\underline{H}_2{}^{13}CHO$), 1.7-1.4 (m, 4 H, $CH(OSi)C\underline{H}_2C\underline{H}_2$), 1.13 (d, 3 H, *J* = 6.2 Hz, $C\underline{H}_3CH(OSi)$), 0.87 (s, 9 H, $(C\underline{H}_3)_3C$), 0.07 (s, 6 H, $(C\underline{H}_3)_2Si$); ^{13}C NMR (90 MHz, $CDCl_3$) δ 202.57 (enriched, $^{13}\underline{C}HO$), 68.16 ($\underline{C}H(OSi)$), 43.87 (d, *J* = 39.1 Hz, $\underline{C}H_2{}^{13}CHO$), 38.99 ($CH(OSi)\underline{C}H_2$), 25.87 ($(\underline{C}H_3)_3C$), 23.69 ($\underline{C}H_3CH(OSi)$), 18.33 ($\underline{C}H_2CH_2{}^{13}CHO$), 18.09 ($(CH_3)_3\underline{C}$), -4.37 and -4.74 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) 232 (MH^+, 100).

For unlabeled racemic material (**125a**): IR (CH_2Cl_2 cast) 2954 (m), 2929 (m), 2857 (m), 1714 (s), 1255 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.77 (t, 1 H, *J* = 1.7 Hz, $C\underline{H}O$), 3.81(ddq, 1 H, *J* = 6.7, 6.1, 5.3 Hz, $C\underline{H}(OSi)$), 2.43 (dt, 2 H, *J* = 7.3, 1.8 Hz, $C\underline{H}_2CHO$), 1.7-1.4 (m, 4 H, $CH(OSi)C\underline{H}_2C\underline{H}_2$), 1.13 (dt, 3 H, *J* = 6.1, 1.6 Hz, $C\underline{H}_3CH(OSi)$), 0.87 (s, 9 H, $(C\underline{H}_3)_3C$), 0.07 (s, 6 H, $(C\underline{H}_3)_2Si$); ^{13}C NMR (90 MHz, $CDCl_3$) δ 202.43 ($\underline{C}HO$), 68.08 ($\underline{C}H(OSi)$), 43.83 ($\underline{C}H_2CHO$), 38.89 ($CH(OSi)\underline{C}H_2$), 25.80 ($(\underline{C}H_3)_3C$), 23.63 ($\underline{C}H_3CH(OSi)$), 18.27 ($\underline{C}H_2CH_2CHO$), 18.01 ($(CH_3)_3\underline{C}$), -4.43 and -4.82 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) 231 (MH^+, 22).

***(S)*-[4,5-$^{13}C_2$]-5-(*tert*-Butyldimethylsiloxy)hexanal (125d).** The method for the conversion of **52d** to **53d** was used. Thus, **124d** (208 mg, 0.887 mmol) gave **125d** (163 mg, 79%). IR (CH_2Cl_2 cast) 2958 (m), 2929 (m), 2857 (m), 1711 (s), 1255 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.77 (t, 1 H, *J* = 1.7 Hz, $C\underline{H}O$), 3.98 and

3.63 (dm, 1 H, J = 140 Hz, $^{13}\mathrm{C\underline{H}(OSi)}$), 2.44 (m, 2 H, $\mathrm{C\underline{H}_2CHO}$), 1.75-1.25 (m, 4 H, $^{13}\mathrm{C\underline{H}_2C\underline{H}_2}$), 1.13 (dt, 3 H, J = 6.0, 4.4 Hz, $\mathrm{C\underline{H}_3{}^{13}CH(OSi)}$), 0.87 (s, 9 H, $\mathrm{(C\underline{H}_3)_3C}$), 0.07 (s, 6 H, $\mathrm{(C\underline{H}_3)_2Si}$); ^{13}C NMR (100 MHz, $\mathrm{CDCl_3}$) δ 202.72 ($\mathrm{\underline{C}HO}$), 68.14 (d, J = 39.3 Hz, enriched, $^{13}\mathrm{\underline{C}H(OSi)}$), 43.89 (d, J = 4.1 Hz, $\mathrm{\underline{C}H_2CHO}$), 38.94 (d, J = 39.2 Hz, enriched, $^{13}\mathrm{\underline{C}H_2}$), 25.86 ($\mathrm{(\underline{C}H_3)_3C}$), 23.70 (t, J = 19.5 Hz, $\mathrm{\underline{C}H_3{}^{13}CH(OSi)}$), 18.33 (t, J = 17.6 Hz, $^{13}\mathrm{CH_2\underline{C}H_2}$), 18.08 ($\mathrm{(CH_3)_3\underline{C}}$), -4.38 and -4.77 ($\mathrm{(\underline{C}H_3)_2Si}$); MS (CI, $\mathrm{NH_3}$) 233 ($\mathrm{MH^+}$, 47).

(*E* and *Z*) Methyl (*S*)-[2,3-$^{13}\mathrm{C}_2$]-7-(*tert*-Butyldimethylsiloxy)oct-2-enoates (126d and 127d). The method used by House *et al.* was followed.[192] In a typical experiment, unlabeled (carbomethoxymethylene)triphenylphosphorane **54a** (802 mg, 2.40 mmol) was added to a solution of unlabeled aldehyde **125a** (460 mg, 2.00 mmol) in MeOH (10 mL) at 20 °C and stirred overnight. Removal of the solvent followed by flash column chromatography (silica, 5% ether in pentane) afforded the *Z*-isomer **127a** (137 mg, 24%), *E*-isomer **126a** (292 mg, 51%), and *Z*+*E* isomers (106 mg, 19%). For the labeled compound, **125c** (472 mg, 2.04 mmol) and [2-^{13}C](carbomethyoxymethylene) triphenylphosphorane **54b** (867 mg, 1.27 mmol) were used; *Z*-isomer **127d** (190 mg, 32%) (R_f 0.58, 5% ether in pentane) and *E*-isomer **126d** (392 mg, 67%) (R_f 0.45), was obtained. For labeled *E*-isomer (**126d**): $[\alpha]_D$ +10.0° (*c* 0.56, $\mathrm{CHCl_3}$); IR ($\mathrm{CHCl_3}$ cast) 2951 (m), 2929 (m), 2857 (m), 1726 (s), 1605 (m) $\mathrm{cm^{-1}}$; ^{1}H NMR (360 MHz, $\mathrm{CDCl_3}$) δ 7.18 and 6.75 (dddt, 1 H, J = 154, 15.6, 6.7, 1.5 Hz, $^{13}\mathrm{C\underline{H}{=}^{13}CHCO}$), 6.04 and 5.59 (ddt, 1 H, J = 162, 15.6, 1.8 Hz, $^{13}\mathrm{CH{=}^{13}C\underline{H}CO}$), 3.78 (m, $\mathrm{C\underline{H}(OSi)}$), 3.73 (s, 3 H, $\mathrm{OC\underline{H}_3}$), 2.18 (m, $\mathrm{C\underline{H}_2{}^{13}CH}$), 1.6 -1.3 (m, 4 H, $\mathrm{CH(OSi)C\underline{H}_2C\underline{H}_2}$), 1.10 (d, 3 H, J = 6.4 Hz, $\mathrm{C\underline{H}_3CH(OSi)}$), 0.87 (s, 9 H, $\mathrm{(C\underline{H}_3)_3C}$), 0.05 (s, 6 H, $\mathrm{(C\underline{H}_3)_2Si}$); ^{13}C NMR (90 MHz, $\mathrm{CDCl_3}$) δ 167.13 (d, J = 74.5 Hz, $\mathrm{\underline{C}OOCH_3}$), 149.42 (d, J = 70.8 Hz, enriched, $^{13}\mathrm{\underline{C}H{=}^{13}CHCO}$), 120.93 (d, J = 70.8 Hz, enriched, $^{13}\mathrm{CH{=}^{13}\underline{C}HCO}$), 68.24 ($\mathrm{\underline{C}H(OSi)}$), 51.33 ($\mathrm{O\underline{C}H_3}$), 39.09 (d, J = 2.5 Hz, $\mathrm{CH(OH)\underline{C}H_2}$), 32.21 (d, J = 41.5 Hz, $\mathrm{\underline{C}H_2{}^{13}CH}$), 25.90 ($(\underline{C}H_3)_3C$), 24.18 (br s, $\underline{C}H_2CH_2{}^{13}CH$), 23.81 ($\underline{C}H_3CH(OSi)$), 18.10 ($(CH_3)_3\underline{C}$), -4.37 and -4.70 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) 289 (MH^+, 65), 306 ($MNH_4{}^+$, 100).

For unlabeled racemic material (*E*-isomer, **126a**): IR ($CHCl_3$ cast) 2952 (m), 2929 (m), 2857 (m), 1728 (s), 1658 (m) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.07 (dt, 1 H, *J* = 15.6, 6.7 Hz, $C\underline{H}$=CHCO), 5.83 (br d, 1 H, *J* = 15.6 Hz, CH=$C\underline{H}$CO), 3.83 (m, $C\underline{H}(OSi)$), 3.78 (s, 3 H, $OC\underline{H}_3$), 2.24 (m, $C\underline{H}_2CH$=CH), 1.6 -1.3 (m, 4 H, $CH(OSi)C\underline{H}_2C\underline{H}_2$), 1.12 (d, 3 H, *J* = 6.4 Hz, $C\underline{H}_3CH(OSi)$), 0.87 (s, 9 H, $(C\underline{H}_3)_3C$), 0.05 (s, 6 H, $(C\underline{H}_3)_2Si$); ^{13}C NMR (90 MHz, $CDCl_3$) δ 167.11 ($\underline{C}OOCH_3$), 149.50 ($\underline{C}H$=CHCO), 120.95 (CH=$\underline{C}$HCO), 68.21 ($\underline{C}H(OSi)$), 51.32 ($O\underline{C}H_3$), 39.05 ($CH(OH)\underline{C}H_2$), 32.23 ($\underline{C}H_2CH$=CH), 25.85 ($(\underline{C}H_3)_3C$), 24.17 (br s, $\underline{C}H_2CH_2CH$=CH), 23.81 ($\underline{C}H_3CH(OSi)$), 18.09 ($(CH_3)_3\underline{C}$), -4.38 and -4.74 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) 287 (MH^+, 91), 304 ($MNH_4{}^+$, 100); Anal. Calcd for $C_{15}H_{30}O_3Si$: C, 62.89; H, 10.56. Found: C, 62.71; H, 10.47.

For labeled *Z*-isomer (**127d**): $[\alpha]_D$ +8.2° (*c* 0.50, $CHCl_3$); IR ($CHCl_3$ cast) 2952 (m), 2929 (m), 1726 (s), 1588 (m) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 6.58 and 5.81 (ddm, 1 H, *J* = 154, 11.4 Hz, $^{13}C\underline{H}$=^{13}CHCO), 6.18 and 5.37 (ddm, 1 H, *J* = 163, 11.4 Hz, ^{13}CH=$^{13}C\underline{H}$CO), 3.78 (m, $C\underline{H}(OSi)$), 3.72 (s, 3 H, $OC\underline{H}_3$), 2.65 (m, $C\underline{H}_2{}^{13}CH$), 1.62 -1.37 (m, 4 H, $CH(OSi)C\underline{H}_2C\underline{H}_2$), 1.12 (d, 3 H, *J* = 6.1 Hz, $C\underline{H}_3CH(OSi)$), 0.87 (s, 9 H, $(C\underline{H}_3)_3C$), 0.07 (s, 6 H, $(C\underline{H}_3)_2Si$); ^{13}C NMR (90 MHz, $CDCl_3$) δ 166.68 (d, *J* = 75.7 Hz, $\underline{C}OOCH_3$), 150.54 (d, *J* = 70.0 Hz, enriched, $^{13}\underline{C}$H=^{13}CHCO), 119.27 (d, *J* = 70.0 Hz, enriched, ^{13}CH=$^{13}\underline{C}$HCO), 68.22 ($\underline{C}H(OSi)$), 50.82 ($O\underline{C}H_3$), 39.13 (d, *J* = 3.7 Hz, $CH(OH)\underline{C}H_2$), 28.82 (d, *J* = 40.3 Hz, $\underline{C}H_2{}^{13}CH$), 25.84 ($(\underline{C}H_3)_3C$), 25.09 (br s, $\underline{C}H_2CH_2{}^{13}CH$), 23.73 ($\underline{C}H_3CH(OSi)$), 18.05 ($(CH_3)_3\underline{C}$), -4.43 and -4.78 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) 289 (MH^+, 100).

For unlabeled racemic material (*Z*-isomer, **127a**): IR ($CHCl_3$ cast) 2953 (m), 2929 (m), 2857 (m), 1727 (s), 1648 (m), 1438 (m) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 6.24 (dt, 1 H, *J* = 11.7, 5.7 Hz, $C\underline{H}$=CHCO), 5.81 (dt, 1 H, *J* = 11.7, 1 7 Hz, CH=$C\underline{H}$CO), 3.86 (m, $C\underline{H}(OSi)$), 3.77 (s, 3 H, $OC\underline{H}_3$), 2.70 (m, 2 H, $C\underline{H}_2CH{=}CH$), 1.6 -1.4 (m, 4 H, $CH(OSi)C\underline{H}_2C\underline{H}_2$), 1.14 (d, 3 H, J = 6.3 Hz, $C\underline{H}_3CH(OSi)$), 0.90 (s, 9 H, $(C\underline{H}_3)_3C$), 0.06 (s, 6 H, $(C\underline{H}_3)_2Si$); ^{13}C NMR (90 MHz, $CDCl_3$) δ 166.80 ($\underline{C}OOCH_3$), 150.71 ($\underline{C}H{=}CHCO$), 119.33 ($CH{=}\underline{C}HCO$), 68.28 ($\underline{C}H(OSi)$), 50.93 ($O\underline{C}H_3$), 39.17 ($CH(OH)\underline{C}H_2$), 28.90 ($\underline{C}H_2CH{=}CH$), 25.88 ($(\underline{C}H_3)_3C$), 25.52 ($\underline{C}H_2CH_2CH{=}CH$), 23.77 ($\underline{C}H_3CH(OSi)$), 18.11 ($(CH_3)_3\underline{C}$), -4.39 and -4.74 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) 287 (MH^+, 87); Anal. Calcd for $C_{15}H_{30}O_3Si$: C, 62.89; H, 10.56. Found: C, 62.54; H, 10.34.

Methyl (*S*)-[6,7-$^{13}C_2$]-7-(*tert*-Butyldimethylsiloxy)oct-2-enoate (126e and 127e). The method for the conversion of **53c** to **55d** was used. Thus, (carbomethoxymethylene)triphenylphosphorane **54a** (890 mg, 2.66 mmol) and the aldehyde **125d** (496 mg, 2.13 mmol) gave **126e** (*E*-isomer, 759 mg, 93%), and **127e** (Z-isomer, 39.7 mg, 4.9%), after flash column chromatography (silica, 5% ether in pentane). For labeled *E*-isomer **126e**: IR (CH_2Cl_2 cast) 2952 (m), 2929 (m), 2857 (m), 1728 (s), 1658 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.97 (dt, 1 H, J = 15.6, 7.0 Hz, $C\underline{H}{=}CHCO$), 5.82 (dt, 1 H, J = 15.6, 1.6 Hz, $CH{=}C\underline{H}CO$), 3.96 and 3.61 (dm, 1 H, J = 140 Hz, $^{13}C\underline{H}(OSi)$), 3.73 (s, 3 H, OCH_3), 2.23 (m, 2 H, $C\underline{H}_2CH{=}CH$), 1.6-1.3 (m, 4 H, $^{13}C\underline{H}_2C\underline{H}_2$), 1.12 (dt, 3 H, J = 6.0, 4.3 Hz, $C\underline{H}_3{}^{13}CH(OSi)$), 0.87 (s, 9 H, $(C\underline{H}_3)_3C$), 0.07 (s, 6 H, $(C\underline{H}_3)_2Si$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.14 ($\underline{C}OOCH_3$), 149.95 (d, J = 2.2 Hz, $\underline{C}H{=}CHCO$), 120.97 ($CH{=}\underline{C}HCO$), 68.23 (d, J = 39.4 Hz, enriched, $^{13}\underline{C}H(OSi)$), 51.34 ($O\underline{C}H_3$), 39.06 (d, J = 39.6 Hz, enriched, $^{13}\underline{C}H_2$), 32.23 (d, J = 4.2 Hz, $\underline{C}H_2CH{=}CH$), 25.88 ($(\underline{C}H_3)_3C$), 24.18 (t, J = 17.8 Hz, $^{13}CH_2\underline{C}H_2$), 23.60 (t, J = 19.6 Hz, $\underline{C}H_3{}^{13}CH(OSi)$), 18.10 ($(CH_3)_3\underline{C}$), -4.37 and -4.73 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) 289 (MH^+, 60), 306 (MNH_4^+, 56).

For labeled Z-isomer **127e**: IR (CH_2Cl_2 cast) 2955 (m), 2929 (m), 2857 (m), 1727 (s), 1642 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.22 (dt, 1 H, J = 11.5, 7.4 Hz, C$\underline{\text{H}}$=CHCO), 5.78 (dt, 1 H, *J* = 11.5, 1.7 Hz, CH=C$\underline{\text{H}}$CO), 3.96 and 3.61 (dm, 1 H, *J* = 140 Hz, ^{13}C$\underline{\text{H}}$(OSi)), 3.71 (s, 3 H, OCH$_3$), 2.65 (m, 2 H, C$\underline{\text{H}}_2$CH=CH), 1.65-1.25 (m, 4 H, ^{13}C$\underline{\text{H}}_2$C$\underline{\text{H}}_2$), 1.12 (dt, 3 H, *J* = 6.0, 4.3 Hz, C$\underline{\text{H}}_3{}^{13}$CH(OSi)), 0.87 (s, 9 H, (C$\underline{\text{H}}_3$)$_3$C), 0.07 (s, 6 H, (C$\underline{\text{H}}_3$)$_2$Si); ^{13}C NMR (100 MHz, CDCl$_3$) δ 166.83 ($\underline{\text{C}}$OOCH$_3$), 150.72 (d, *J* = 3.7 Hz, $\underline{\text{C}}$H=CHCO), 119.35 (CH=$\underline{\text{C}}$HCO), 68.29 (d, *J* = 38.7 Hz, enriched, $^{13}\underline{\text{C}}$H(OSi)), 50.94 (O$\underline{\text{C}}H_3$), 39.05 (d, *J* = 39.0 Hz, enriched, $^{13}\underline{\text{C}}H_2$), 28.92 (d, *J* = 4.5 Hz, $\underline{\text{C}}$H$_2$CH=CH), 25.89 (($\underline{\text{C}}$H$_3$)$_3$C), 25.15 (t, *J* = 17.5 Hz, ^{13}CH$_2\underline{\text{C}}$H$_2$), 23.77 (t, *J* = 19.6 Hz, $\underline{\text{C}}$H$_3{}^{13}$CH(OSi)), 18.12 ((CH$_3$)$_3\underline{\text{C}}$), -4.39 and -4.74 (($\underline{\text{C}}$H$_3$)$_2$Si); MS (CI, NH$_3$) 289 (MH$^+$, 72).

Methyl *(S)*-[6,7-^{13}C$_2$,7-*siloxy*-^{18}O]-7-(*tert*-Butyldimethylsiloxy)oct-2-enoate (126f). The method for the conversion of **40c** to **41c** was used. Thus, **134f** (137 mg, 0.777 mmol) afforded **126f** (163 mg, 72%). IR (CH$_2$Cl$_2$ cast) 2952 (m), 2929 (m), 2857 (m), 1728 (s), 1568 (m) cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 6.97 (dt, 1 H, *J* = 15.6, 7.0 Hz, C$\underline{\text{H}}$=CHCO), 5.82 (dt, 1 H, *J* = 15.6, 1.3 Hz, CH=C$\underline{\text{H}}$CO), 3.91 and 3.61 (dm, 1 H, *J* = 140 Hz, ^{13}C$\underline{\text{H}}$($^{18/16}$OSi)), 2.23 (m, 2 H, C$\underline{\text{H}}_2$CH=CH), 1.6-1.3 (m, 4 H, ^{13}C$\underline{\text{H}}_2$C$\underline{\text{H}}_2$), 1.12 (dt, 3 H, *J* = 6.0, 4.4 Hz, C$\underline{\text{H}}_3{}^{13}$CH($^{18/16}$OSi)), 0.87 (s, 9 H, (C$\underline{\text{H}}_3$)$_3$C), 0.07 (s, 6 H, (C$\underline{\text{H}}_3$)$_2$Si); ^{13}C NMR (100 MHz, CDCl$_3$) δ 167.18 ($\underline{\text{C}}$OOCH$_3$), 149.58 (d, *J* = 3.0 Hz, $\underline{\text{C}}$H=CHCO), 120.93 (CH=$\underline{\text{C}}$HCO), 68.20 (dd, *J* = 39.2, 3.0 Hz, enriched, $^{13}\underline{\text{C}}$H($^{18/16}$OSi)), 51.38 (O$\underline{\text{C}}H_3$), 39.11 (d, *J* = 39.4 Hz, enriched, $^{13}\underline{\text{C}}H_2$), 32.24 (d, *J* = 4.3 Hz, $\underline{\text{C}}$H$_2$CH=CH), 25.88 (($\underline{\text{C}}$H$_3$)$_3$C), 24.19 (t, *J* = 17.5 Hz, ^{13}CH$_2\underline{\text{C}}$H$_2$), 24.01 (t, *J* = 19.3 Hz, $\underline{\text{C}}$H$_3{}^{13}$CH($^{18/16}$OSi)), 18.11 ((CH$_3$)$_3\underline{\text{C}}$), -4.37 and -4.73 (($\underline{\text{C}}$H$_3$)$_2$Si); MS (CI, NH$_3$) 289 (MH$^+$(^{16}O), 5.9), 291 (MH$^+$(^{18}O), 9.3).

(*S*)-[1,2-^{13}C$_2$]-7-(*tert*-Butyldimethylsiloxy)oct-2-enoic Acid (128d). The method for the conversion of **55d** to **56d** was used. Thus, **126d** (422 mg, 1.46 mmol) afforded **128d** (401 mg, 99%), which was used directly in the next reaction without further purification. IR ($\mathrm{CHCl_3}$ cast) 3400-2400 (br), 2954 (m), 2929 (m), 2857 (m), 1695 (s), 1603 (m) $\mathrm{cm^{-1}}$; ^{1}H NMR (360 MHz, $\mathrm{CDCl_3}$) δ 7.29 and 6.89 (dddt, 1 H, *J* = 154, 16, 6.7, 1.8 Hz, $^{13}\mathrm{C}\underline{\mathrm{H}}{=}^{13}\mathrm{CHCO}$), 6.06 and 5.61 (ddt, 1 H, *J* = 163, 16, 6.1 Hz, $^{13}\mathrm{CH}{=}^{13}\mathrm{C}\underline{\mathrm{H}}\mathrm{CO}$), 3.81 (m, $\mathrm{C}\underline{\mathrm{H}}\mathrm{(OSi)}$), 2.22 (m, 2 H, $\mathrm{C}\underline{\mathrm{H}}_2{}^{13}\mathrm{CH}$), 1.62 -1.34 (m, 4 H, $\mathrm{CH(OSi)C}\underline{\mathrm{H}}_2\mathrm{C}\underline{\mathrm{H}}_2$), 1.13 (d, 3 H, *J* = 6.4 Hz, $\mathrm{C}\underline{\mathrm{H}}_3\mathrm{CH(OSi)}$), 0.89 (s, 3 H, $\mathrm{(C}\underline{\mathrm{H}}_3)_3\mathrm{C}$), 0.06 (s, 6 H, $\mathrm{(C}\underline{\mathrm{H}}_3)_2\mathrm{Si}$); ^{13}C NMR (90 MHz, $\mathrm{CDCl_3}$) δ 171.75 (d, *J* = 72 Hz, $\underline{\mathrm{C}}\mathrm{OOH}$), 152.04 (d, *J* = 70.0 Hz, enriched, $^{13}\underline{\mathrm{C}}\mathrm{H}{=}^{13}\mathrm{CHCO}$), 120.73 (d, *J* = 70.0 Hz, enriched, $^{13}\mathrm{CH}{=}^{13}\underline{\mathrm{C}}\mathrm{HCO}$), 68.22 ($\underline{\mathrm{C}}\mathrm{H(OSi)}$), 39.26 ($\mathrm{CH(OH)}\underline{\mathrm{C}}\mathrm{H_2}$), 32.33 (d, *J* = 41.5 Hz, $\underline{\mathrm{C}}\mathrm{H_2}{}^{13}\mathrm{CH}$), 25.88 ($\mathrm{((}\underline{\mathrm{C}}\mathrm{H_3)_3C}$), 24.04 (br s, $\underline{\mathrm{C}}\mathrm{H_2CH_2}{}^{13}\mathrm{CH}$), 23.25 ($\underline{\mathrm{C}}\mathrm{H_3CH(OSi)}$), 18.11 ($\mathrm{((CH_3)_3}\underline{\mathrm{C}}$), -4.37 and -4.73 ($\mathrm{((}\underline{\mathrm{C}}\mathrm{H_3)_2Si}$); MS (CI, $\mathrm{NH_3}$) 275 ($\mathrm{MH^+}$, 98).

For unlabeled racemic material (**128a**, *E*-isomer): IR ($\mathrm{CHCl_3}$ cast) 3400-2400 (br), 2955 (m), 2929 (m), 2848 (m), 1699 (s), 1657 (m) $\mathrm{cm^{-1}}$; ^{1}H NMR (360 MHz, $\mathrm{CDCl_3}$) δ 7.07 (dt, 1 H, *J* = 15.6, 7 Hz, $\mathrm{C}\underline{\mathrm{H}}\mathrm{{=}CHCO}$), 5.83 (dt, 1 H, *J* = 15.6, 1.5 Hz, $\mathrm{CH{=}C}\underline{\mathrm{H}}\mathrm{CO}$), 3.80 (m, $\mathrm{C}\underline{\mathrm{H}}\mathrm{(OSi)}$), 2.35 (m, $\mathrm{C}\underline{\mathrm{H}}_2\mathrm{CH{=}CH}$), 1.62 -1.34 (m, 4 H, $\mathrm{CH(OSi)C}\underline{\mathrm{H}}_2\mathrm{C}\underline{\mathrm{H}}_2$), 1.13 (d, 3 H, *J* = 6 Hz, $\mathrm{C}\underline{\mathrm{H}}_3\mathrm{CH(OSi)}$), 0.89 (s, 9 H, $\mathrm{(C}\underline{\mathrm{H}}_3)_3\mathrm{C}$), 0.06 (s, 6 H, $\mathrm{(C}\underline{\mathrm{H}}_3)_2\mathrm{Si}$); ^{13}C NMR (90 MHz, $\mathrm{CDCl_3}$) δ 171.46 ($\underline{\mathrm{C}}\mathrm{OOH}$), 152.17 ($\underline{\mathrm{C}}\mathrm{H{=}CHCO}$), 120.62 ($\mathrm{CH{=}}\underline{\mathrm{C}}\mathrm{HCO}$), 68.21 ($\underline{\mathrm{C}}\mathrm{H(OSi)}$), 39.03 ($\mathrm{CH(OH)}\underline{\mathrm{C}}\mathrm{H_2}$), 32.34 ($\underline{\mathrm{C}}\mathrm{H_2CH{=}CH}$), 25.87 ($\mathrm{((}\underline{\mathrm{C}}\mathrm{H_3)_3C}$), 24.05 ($\underline{\mathrm{C}}\mathrm{H_2CH_2CH{=}CH}$), 23.82 ($\underline{\mathrm{C}}\mathrm{H_3CH(OSi)}$), 18.11 ($\mathrm{((CH_3)_3}\underline{\mathrm{C}}$), -4.37 and -4.74 ($\mathrm{((}\underline{\mathrm{C}}\mathrm{H_3)_2Si}$); MS (CI, $\mathrm{NH_3}$) 273 ($\mathrm{MH^+}$, 42), 287 ($\mathrm{MNH_4^+}$, 72); Anal. Calcd for $\mathrm{C_{14}H_{28}O_3Si}$: C, 61.72; H, 10.36. Found: C, 61.71; H, 10.41.

For unlabeled racemic material (*Z*-isomer, **129a**): IR ($\mathrm{CHCl_3}$ cast) 3400-2400 (br), 2956 (m), 2930 (m), 2857 (m), 1699 (s), 1641 (m) $\mathrm{cm^{-1}}$; ^{1}H NMR (360 MHz, $\mathrm{CDCl_3}$) δ 6.34 (dt, 1 H, *J* = 11.5, 7.5 Hz, $\mathrm{C}\underline{\mathrm{H}}\mathrm{{=}CHCO}$), 5.80 (dt, 1 H, *J* = 11.5, 1.8 Hz, $\mathrm{CH{=}C}\underline{\mathrm{H}}\mathrm{CO}$), 3.80 (m, $\mathrm{C}\underline{\mathrm{H}}\mathrm{(OSi)}$), 2.66 (m, 2 H, $\mathrm{C}\underline{\mathrm{H}}_2\mathrm{CH{=}CH}$), 1.6 -1.4 (m, 4 H, $\mathrm{CH(OSi)C}\underline{\mathrm{H}}_2\mathrm{C}\underline{\mathrm{H}}_2$), 1.12 (d, 3 H, *J* = 6.2 Hz, $\mathrm{C}\underline{\mathrm{H}}_3\mathrm{CH(OSi)}$), 0.87 (s, 9 H, $\mathrm{(C}\underline{\mathrm{H}}_3)_3\mathrm{C}$), 0.06 (s, 6 H, $\mathrm{(C}\underline{\mathrm{H}}_3)_2\mathrm{Si}$); ^{13}C NMR (90 MHz, $\mathrm{CDCl_3}$) δ 171.61 ($\underline{\mathrm{C}}\mathrm{OOH}$), 153.26

($\underline{C}H{=}CHCO$), 119.15 ($CH{=}\underline{C}HCO$), 68.25 ($\underline{C}H(OSi)$), 39.12 ($CH(OH)\underline{C}H_2$), 29.10 ($\underline{C}H_2CH{=}CH$), 25.88 ($(\underline{C}H_3)_3C$), 25.08 ($\underline{C}H_2CH_2CH{=}CH$), 23.79 ($\underline{C}H_3CH(OSi)$), 18.11 ($(CH_3)_3\underline{C}$), -4.40 and -4.76 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) 273 (MH^+, 100), 287 (MNH_4^+, 41); Anal. Calcd for $C_{14}H_{28}O_3Si$: C, 61.72; H, 10.36. Found: C, 61.67; H, 10.21.

(*S*)-[6,7-$^{13}C_2$,7-siloxy-^{18}O]-7-(tert-Butyldimethylsiloxy)oct-2-enoic Acid (**128f**). The method for the conversion of **55d** to **56d** was used. Thus, **126f** (190 mg, 0.655 mmol) gave **128f** (180 mg, crude), which was used directly for the next reaction.

NAC (*S*)-[1,2-$^{13}C_2$]-7-(*tert*-Butyldimethylsiloxy)oct-2-enoate (130d). The method for the conversion of **56d** to **57d** was used. Thus, crude **128d** (401 mg, 1.46 mmol) gave **130d** (309 mg, 56%), after chromatographic purification (silica, EtOAc, R_f 0.49). $[\alpha]_D$ +6.5° (*c* 0.26, $CHCl_3$); IR ($CHCl_3$ cast) 3283 (br), 2929 (m), 1657 (s), 1583 (m), 1558 (m) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.20 and 6.72 (dm, 1 H, J = 154 Hz, $^{13}C\underline{H}{=}^{13}CHCO$), 6.42 and 6.00 (dm, 1 H, J = 160 Hz, $^{13}CH{=}^{13}C\underline{H}CO$), 6.10 (br s, 1 H, $N\underline{H}$), 3.85 (m, $C\underline{H}(OSi)$), 3.60 (dt, 2 H, J = 6.4, 5.4 Hz, $C\underline{H}_2NH$), 3.21 (t, 2 H, J = 6.4 Hz, $SC\underline{H}_2$), 2.37 (m, $C\underline{H}_2{}^{13}CH$), 2.11 (s, 3 H, $COC\underline{H}_3$), 1.75 -1.6 (m, 4 H, $CH(OSi)C\underline{H}_2C\underline{H}_2$), 1.23 (d, 3 H, J = 6.4 Hz, $C\underline{H}_3CH(OSi)$), 0.90 (s, 9 H, $(C\underline{H}_3)_3C$), 0.10 (s, 6 H, $(C\underline{H}_3)_2Si$); ^{13}C NMR (90 MHz, $CDCl_3$) δ 190.33 (d, J = 61.0 Hz, $\underline{C}OS$), 170.23 ($\underline{C}OCH_3$), 146.47 (d, J = 70.0 Hz, enriched, $^{13}\underline{C}H{=}^{13}CHCO$), 128.83 (d, J = 70.0 Hz, enriched, $^{13}CH{=}^{13}\underline{C}HCO$), 68.17 ($\underline{C}H(OSi)$), 39.82 ($CO\underline{C}H_3$), 39.01 (d, J = 3.7 Hz, $CH(OH)\underline{C}H_2$), 32.22 (d, J = 40.3 Hz, $\underline{C}H_2{}^{13}CH$), 26.61 ($S\underline{C}H_2$), 25.87 ($(\underline{C}H_3)_3C$), 24.01 (d, J = 2.4 Hz, $\underline{C}H_2CH_2{}^{13}CH$), 23.77 ($\underline{C}H_3CH(OSi)$), 23.20 ($\underline{C}H_2NH$), 15.69 ($(CH_3)_3\underline{C}$), -4.38 and -4.73 ($(\underline{C}H_3)_2Si$); MS (EI) calcd for $^{13}C_2C_{16}H_{35}NO_3SSi$ 375.2174 , found 375.2155 (M); MS (CI, NH_3) 376 (MH^+, 100).

For unlabeled racemic material (**130a**): IR ($CHCl_3$ cast) 3325 (br), 2929 (m), 1663 (s), 1632 (m), 1557 (m) cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 6.94 (dt, 1 H, *J* = 15.6 Hz, $C\underline{H}=CHCO$), 6.13 (dt, 1 H, *J* = 15.6 Hz, $CH=C\underline{H}CO$), 5.94 (br s, 1 H, $N\underline{H}$), 3.78 (m, $C\underline{H}(OSi)$), 3.45 (dt, 2 H, *J* = Hz, $C\underline{H}_2NH$), 3.08 (t, 2 H, *J* = Hz, $SC\underline{H}_2$), 2.22 (m, 2 H, $C\underline{H}_2CH=CH$), 1.97 (s, 3 H, $COC\underline{H}_3$), 1.7-1.4 (m, 4 H, $CH(OSi)C\underline{H}_2C\underline{H}_2$), 1.12 (d, 3 H, *J* = Hz, $C\underline{H}_3CH(OSi)$), 0.90 (s, 9 H, $(C\underline{H}_3)_3C$), 0.10 (s, 6 H, $(C\underline{H}_3)_2Si$); ^{13}C NMR (90 MHz, $CDCl_3$) δ 190.328 ($\underline{C}OS$), 170.28 ($\underline{C}OCH_3$), 146.42 ($\underline{C}H=CHCO$), 128.32 ($CH=\underline{C}HCO$), 68.11 ($\underline{C}H(OSi)$), 39.73 ($CO\underline{C}H_3$), 38.97 ($CH(OH)\underline{C}H_2$), 32.20 ($\underline{C}H_2CH=CH$), 28.17 ($S\underline{C}H_2$), 25.81 ($(\underline{C}H_3)_3C$), 23.99 ($\underline{C}H_2CH_2CH=CH$), 23.73 ($\underline{C}H_3CH(OSi)$), 23.14 ($\underline{C}H_2NH$), 18.03 ($(CH_3)_3\underline{C}$), -4.43 and -4.79 ($(\underline{C}H_3)_2Si$); MS (EI) calcd for $C_{18}H_{35}NO_3SSi$ 373.2107, found 373.2083 (M); Anal. Calcd for $C_{18}H_{35}NO_3SSi$: C, 57.86; H, 9.44; N, 3.75; S, 8.58. Found: C, 58.12; H, 9.40; N, 3.70; S, 8.41.

NAC (*S*)-[6,7-$^{13}C_2$,7-*siloxy*-^{18}O]-7-(*tert*-Butyldimethylsiloxy)oct-2-enoate (130f). The method for the conversion of **56d** to **57d** was used. Thus, **128f** (180 mg, 0.655 mmol) afforded **130f** (59.1 mg, 24%). IR (CH_2Cl_2 cast) 3288 (br), 2929 (m), 2887 (m), 1656 (s), 1552 (m) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.93 (dt, 1 H, *J* = 15.6, 7.0 Hz, $C\underline{H}=CHCO$), 6.15 (dt, 1 H, *J* = 15.6, 1.5 Hz, $CH=C\underline{H}CO$), 5.87 (br s, 1 H, $N\underline{H}$), 3.98 and 3.63 (dm, 1 H, *J* = 141 Hz, $^{13}C\underline{H}(^{18/16}OSi)$), 3.46 (q, 2 H, *J* = 6.2 Hz, $C\underline{H}_2NH$), 3.09 (t, 2 H, *J* = 6.3 Hz, $SC\underline{H}_2$), 2.21 (m, 2 H, $C\underline{H}_2CH=CH$), 1.97 (s, 3 H, $COC\underline{H}_3$), 1.7-1.3 (m, 4 H, $^{13}C\underline{H}_2C\underline{H}_2$), 1.21 (dt, 3 H, *J* = 6.0 Hz, 4.4 Hz, $C\underline{H}_3{}^{13}CH(^{18/16}OSi)$), 0.87 (s, 9 H, $(C\underline{H}_3)_3C$), 0.07 (s, 6 H, $(C\underline{H}_3)_2Si$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.37 ($\underline{C}OS$), 170.28 ($\underline{C}OCH_3$), 146.51 (d, *J* = 3.0 Hz, $\underline{C}H=CHCO$), 128.35 ($CH=\underline{C}HCO$), 68.12 (br d, *J* = 39.9 Hz, enriched, $^{13}\underline{C}H(^{18/16}OSi)$), 39.56 ($CO\underline{C}H_3$), 39.00 (d, *J* = 39.3 Hz, enriched, $^{13}\underline{C}H_2$), 32.23 (d, *J* = 4.3 Hz, $\underline{C}H_2CH=CH$), 28.21 ($S\underline{C}H_2$), 25.84 ($(\underline{C}H_3)_3C$), 24.00 (t, *J* = 17.5 Hz, $^{13}CH_2\underline{C}H_2$), 23.76 (t, *J* = 19.6

Hz, $\underline{C}H_3{}^{13}CH({}^{18/16}OSi)$), 23.18 ($\underline{C}H_2NH$), 18.07 ($(CH_3)_3\underline{C}$), -4.40 and -4.76 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) 376 ($MH^+({}^{16}O)$, 8.8), 378 ($MH^+({}^{18}O)$, 19.1).

2,2,2-Trichloroethylbutyrate (131). Trichloroethanol (45.0g, 301 mmol) was added dropwise to liquid butyryl chloride (26.6 g, 250 mmol) over 25 min at 0 °C. The mixture was allowed to warm to room temperature and was stirred overnight. Vacuum distillation gave **131** in quantitative yield. Bp 69-72 °C (2.8 mm Hg); IR ($CHCl_3$ cast) 2960 (m), 2929 (m), 2858 (m), 1757 (s) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.75 (s, 2 H, $C\underline{H}_2CCl_3$), 2.43 (t, 2 H, J = 7.5 Hz, $C\underline{H}_2COO$), 1.72 (tq, 2 H, J = 7.5, 7.5 Hz, $CH_3C\underline{H}_2$), 1.01 (t, 3H, J = 7.5 Hz, $C\underline{H}_3$); ^{13}C NMR (90 MHz, $CDCl_3$) δ 171.95 ($\underline{C}O$), 95.05 ($\underline{C}Cl_3$), 73.82 ($O\underline{C}H_2$), 35.79 ($\underline{C}H_2CO$), 18.27 ($CH_3\underline{C}H_3$), 13.58 ($\underline{C}H_3$); MS (EI) calcd for $C_6H_9Cl_3O_2$ 217.9668, found 217.9673 (M).

2,2,2-Trichlorolauroate (132). The method for preparation of **131** was adapted. Thus, trichloroethanol (35.9 g, 240 mmol) and lauroyl chloride (43.7 g, 200 mmol) gave **132** in quantitative yield. Bp 140-142 °C (0.2 mm Hg); IR ($CHCl_3$ cast) 2954 (m), 2925 (m), 2855 (m), 1759 (s) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.75 (s, 2 H, $C\underline{H}_2CCl_3$), 2.48 (t, 2 H, J = 7.5 Hz, $C\underline{H}_2COO$), 1.70 (m, 2 H), 1.23 (m, 16 H), 0.88 (m, 3H, $C\underline{H}_3$); MS (EI) calcd for $C_{14}H_{25}Cl_3O_2$ 330.0920, found 330.0925 (M).

Methyl *(S)*-[6,7-$^{13}C_2$]-7-Hydroxyoct-2-enoate (134d). The method for the conversion of **130d** to **82d** was used. Thus, the silyl ether **126e** (753 mg, 2.61 mmol) afforded **134d** (394 mg, 87%) along with recovery of **126e** (93.0 mg, 12%), after column chromatography (silica, EtOAc, R_f 0.71, R_f 0.92, respectively). IR (CH_2Cl_2 cast) 3400 (m), 2929 (m), 1725 (s), 1658 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.97 (dt, 1 H, J = 15.6, 7.0 Hz, $C\underline{H}=CHCO$), 5.83 (dt, 1 H, J = 15.6, 1.5 Hz, $CH=C\underline{H}CO$), 3.98 and 3.63 (dm, 1 H, J = 140 Hz, $^{13}C\underline{H}(OH)$), 3.73 (s, 3 H, OCH_3), 2.24 (m, 2 H,

$\mathrm{C\underline{H}_2CH{=}CH}$), 1.6-1.3 (m, 4 H, $\mathrm{^{13}C\underline{H}_2C\underline{H}_2}$), 1.20 (dt, 3 H, *J* = 6.2, 4.4 Hz, $\mathrm{C\underline{H}_3{}^{13}CH(OH)}$); ^{13}C NMR (100 MHz, $\mathrm{CDCl_3}$) δ 167.07 ($\mathrm{\underline{C}OOCH_3}$), 149.13 (d, *J* = 4.0 Hz, $\mathrm{\underline{C}H{=}CHCO}$), 121.20 ($\mathrm{CH{=}\underline{C}HCO}$), 67.78 (d, *J* = 37.9 Hz, enriched, $\mathrm{^{13}\underline{C}H(OH)}$), 51.36 ($\mathrm{O\underline{C}H_3}$), 38.99 (d, *J* = 38.1 Hz, enriched, $\mathrm{^{13}\underline{C}H_2}$), 32.06 (d, *J* = 4.4 Hz, $\mathrm{\underline{C}H_2CH{=}CH}$), 24.19 (t, *J* = 17.6 Hz, $\mathrm{^{13}CH_2\underline{C}H_2}$), 23.60 (t, *J* = 19.3 Hz, $\mathrm{\underline{C}H_3{}^{13}CH(OH)}$); MS (CI, $\mathrm{NH_3}$) 175 ($\mathrm{MH^+}$, 31), 192 ($\mathrm{MNH_4{}^+}$, 100).

For unlabeled racemic material (**134a**): IR ($\mathrm{CH_2Cl_2}$ cast) 3400 (m), 2929 (m), 1725 (s), 1658 (m) $\mathrm{cm^{-1}}$; ^{1}H NMR (400 MHz, $\mathrm{CDCl_3}$) δ 6.97 (dt, 1 H, *J* = 15.6, 7.0 Hz, $\mathrm{C\underline{H}{=}CHCO}$), 5.83 (dt, 1 H, *J* = 15.6, 1.5 Hz, $\mathrm{CH{=}C\underline{H}CO}$), 3.82 (m, 1 H, $\mathrm{C\underline{H}(OH)}$), 3.73 (s, 3 H, $\mathrm{OCH_3}$), 2.25 (m, 2 H, $\mathrm{C\underline{H}_2CH{=}CH}$), 1.6-1.3 (m, 4 H, $\mathrm{CH(OH)C\underline{H}_2C\underline{H}_2}$), 1.20 (d, 3 H, *J* = 6.3, $\mathrm{C\underline{H}_3CH(OH)}$); ^{13}C NMR (100 MHz, $\mathrm{CDCl_3}$) δ 167.07 ($\mathrm{\underline{C}OOCH_3}$), 149.14 ($\mathrm{\underline{C}H{=}CHCO}$), 121.18 ($\mathrm{CH{=}\underline{C}HCO}$), 67.79 ($\mathrm{\underline{C}H(OH)}$), 51.37 ($\mathrm{O\underline{C}H_3}$), 38.58 ($\mathrm{CH(OH)\underline{C}H_2}$), 32.06 ($\mathrm{\underline{C}H_2CH{=}CH}$), 24.19 ($\mathrm{CH(OH)CH_2\underline{C}H_2}$), 23.61 ($\mathrm{\underline{C}H_3CH(OH)}$); MS (CI, $\mathrm{NH_3}$) 173 ($\mathrm{MH^+}$, 67), 190 ($\mathrm{MNH_4{}^+}$, 100).

Racemic Methyl [6,7-^{13}C$_2$,7-*hydroxy*-^{18}O]-7-Hydroxyoct-2-enoate (134e). The method for the conversion of **39d** to **40e** was used. Thus, the keto compound **135f** (1.96 mmol, based on 100% conversion) afforded **134f** (309 mg, 89%). IR ($\mathrm{CH_2Cl_2}$ cast) 3400 (m), 2929 (m), 1725 (s), 1658 (m) $\mathrm{cm^{-1}}$; ^{1}H NMR (400 MHz, $\mathrm{CDCl_3}$) δ 6.97 (dt, 1 H, *J* = 15.6, 7.0 Hz, $\mathrm{C\underline{H}{=}CHCO}$), 5.83 (dt, 1 H, *J* = 15.6, 1.5 Hz, $\mathrm{CH{=}C\underline{H}CO}$), 3.98 and 3.63 (dm, 1 H, *J* = 140 Hz, $\mathrm{^{13}C\underline{H}(^{18/16}OH)}$), 3.73 (s, 3 H, $\mathrm{OCH_3}$), 2.24 (m, 2 H, $\mathrm{C\underline{H}_2CH{=}CH}$), 1.6-1.3 (m, 4 H, $\mathrm{^{13}C\underline{H}_2C\underline{H}_2}$), 1.20 (dt, 3 H, *J* = 6.2, 4.4 Hz, $\mathrm{C\underline{H}_3{}^{13}CH(^{18/16}OH)}$); ^{13}C NMR (100 MHz, $\mathrm{CDCl_3}$) δ 167.07 ($\mathrm{\underline{C}OOCH_3}$), 149.23 (d, *J* = 2.3 Hz, $\mathrm{\underline{C}H{=}CHCO}$), 121.06 ($\mathrm{CH{=}\underline{C}HCO}$), 67.62 (d, *J* = 38.1 Hz, enriched, $\mathrm{^{13}\underline{C}H(^{18/16}OH)}$), 51.34 ($\mathrm{O\underline{C}H_3}$), 38.50 (d, *J* = 38.3 Hz, enriched, $\mathrm{^{13}\underline{C}H_2}$), 32.02 (d, *J* = 4.4 Hz, $\mathrm{\underline{C}H_2CH{=}CH}$), 24.12 (t, *J* = 17.5 Hz, $\mathrm{^{13}CH_2\underline{C}H_2}$), 23.51 (t, *J* =

19.1 Hz, $\underline{C}H_3{}^{13}CH(OH)$); MS (CI, NH_3) 175 (MH^+(^{16}O), 10.7), 177 (MH^+(^{18}O), 37.5), 192 (MNH_4^+(^{16}O), 15.3), 194 (MNH_4^+(^{18}O), 56.6).

Methyl *(S)*-[6,7-$^{13}C_2$, *7-hydroxy*-^{18}O]-7-Hydroxyoct-2-enoate (134f). The method for the conversion of **40e** to **40f** was used. Thus, **134e** (288 mg, 1.70 mmol) gave the hydroxy compound **134f** (140.6 mg, 49%). IR, MS, ^1H NMR and ^{13}C NMR were identical to the racemic material **134e**.

Methyl [6,7-$^{13}C_2$]-7-Oxo-2-octenoate (135d). The method for the conversion of **52d** to **53d** was used. Thus, the hydroxy compound **134d** (387 mg, 2.22 mmol) gave the keto compound **135d** (343 mg, 90%, R_f 0.78 in EtOAc). IR (CH_2Cl_2, cast) 2929 (m), 1723 (s), 1674, 1271 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.93 (dt, 1 H, J = 15.6, 7.0 Hz, $C\underline{H}$=CHCO), 5.84 (dt, 1 H, J = 15.6, 1.5 Hz, CH=$C\underline{H}$CO), 3.73 (s, 3 H, OCH_3), 2.62 and 2.31 (ddt, 2 H, J = 126, 7.2, 5.5 Hz, $^{13}C\underline{H}_2$), 2.23 (m, 2 H, $C\underline{H}_2$CH=CH), 2.14 (dd, 3 H, J = 5.8, 1.4 Hz, $C\underline{H}_3{}^{13}CO$), 1.75 (ddt, 2 H, J = 14.7, 7.3, 3.7 Hz, $^{13}CH_2C\underline{H}_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.97 (d, J = 39.0 Hz, enriched, ^{13}CO), 166.39 ($\underline{C}OOCH_3$), 148.32 (d, J = 2.7 Hz, $\underline{C}$H=CHCO), 121.65 (CH=$\underline{C}$HCO), 51.42 ($O\underline{C}H_3$), 42.54 (d, J = 39.6 Hz, enriched, $^{13}\underline{C}H_2$), 31.32 (d, J = 3.5 Hz, $\underline{C}H_2$CH=CH), 29.78 (t, J = 17.8 Hz, $\underline{C}H_3{}^{13}CH(OH)$), 21.88 (t, J = 18.0 Hz, $^{13}CH_2\underline{C}H_2$); MS (CI, NH_3) 173 (MH^+, 38), 190 (MNH_4^+, 100).

For unlabeled material (**135a**): IR (CH_2Cl_2 cast) 2929 (m), 1718 (s), 1658, 1272 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.93 (dt, 1 H, J = 15.6, 7.0 Hz, $C\underline{H}$=CHCO), 5.84 (dt, 1 H, J = 15.6, 1.5 Hz, CH=$C\underline{H}$CO), 3.73 (s, 3 H, OCH_3), 2.46 (t, 2 H, J = 7.3 Hz, $COC\underline{H}_2$), 2.22 (dq, 2 H, J = 7.2, 1.5 Hz, $C\underline{H}_2$CH=CH), 2.16 (s, 3 H, $C\underline{H}_3CO$), 1.75 (tt, 2 H, J = 14.6, 7.3 Hz, $COCH_2C\underline{H}_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 208.05 ($CH_3\underline{C}O$), 166.92 ($\underline{C}OOCH_3$), 148.34 ($\underline{C}$H=CHCO), 121.59 (CH=$\underline{C}$HCO), 51.43

($O\underline{C}H_3$), 42.51 ($CO\underline{C}H_2$), 31.30 ($\underline{C}H_2CH{=}CH$), 29.96 ($\underline{C}H_3CH(OH)$), 21.82 ($COCH_2\underline{C}H_2$); MS (EI) calcd for $C_9H_{14}O_3$ 170.0943, found 170.0947 (M).

Methyl [6,7-$^{13}C_2$,7-oxo-^{18}O]-7-Oxo-2-octenoate (**135e**). A modification of the procedure of Diakur was used.[194] A mixture of the keto compound **135d** (338 mg, 1.96 mmol), $H_2{}^{18}O$ (97%, Cambridge Isotope Laboratory, 500 mg, 25.0 mmol), THF (2 mL), and trifluoroacetic anhydride (10 μL) was heated at 70 °C for 9 h. The mixture was transferred to a separatory funnel and the organic phase was collected, dried (Na_2SO_4), and concentrated to give **135e**, which was used immediately for the next reaction.

REFERENCES

(1) (a) Collie, J. N. *J. Chem. Soc.* **1907**, *91*, 1806-1813. (b) Collie, J. N.; Myers, W. S. *J. Chem. Soc.* **1893**, *63*, 122-128.

(2) (a) Birch, A. J.; Donovan, F. W. *Aust. J. Chem.* **1953**, *6*, 360-368. (b) Birch, A. J. *Science* **1967**, *156*, 202-206.

(3) Birch, A. J.; Massy-Westropp, R. A.; Moye, C. J. *Aust. J. Chem.* **1955**, *8*, 539-544.

(4) For reviews see: (a) Vederas, J. C. *Nat. Prod. Rep.* **1987**, *4*, 277-337. (b) Simpson, T. J. *Chem. Soc. Rev.* **1987**, *16*, 123-160. (c) Simpson, T. J. *Nat. Prod. Rep.* **1987**, *4*, 339-376. (d) O'Hagan, D. *Nat. Prod. Rep.* **1989**, *6*, 205-219.

(5) Lynen, F. *Fed. Proc.* **1961**, *20*, 941-951.

(6) Scott, A. I.; Wiesner, C. J.; Yoo, S.; Chung, S.-K. *J. Am. Chem. Soc.* **1975**, *97*, 6277-6278.

(7) Kobuke, Y.; Yoshida, J.-I. *Tetrahedron Lett.* **1978**, 367-370.

(8) For leading references see: (a) 6-Methylsalicylic acid synthase: Lam, K. S.; Neway, J. O.; Gaucher, G. M. *Can. J. Microbiol.* **1988**, *34*, 30-37. (b) Orsellinate synthase: Woo, E.-R.; Fujii, I.; Ebizuka, Y.; Sankawa, U.; Kawaguchi, A.; Huang, S.; Beale, J. M.; Shibuya, M.; Mocek, U.; Floss, H. G. *J. Am. Chem Soc.* **1989**, *111*, 5498-5500. (c) Chalcone synthases: Schroeder, J.; Schroeder, G. *Z. Naturforsch., C: Biosci.* **1990**, *45*, 1-8.

(9) Aldridge, D. C.; Galt, S.; Giles, D.; Turner, W. B. *J. Chem. Soc. C* **1971**, 1623-1627.

(10) Oyama, H.; Sassa, T.; Ikeda, M. *Agric. Biol. Chem.* **1978**, *42*, 2407-1409.

(11) Lai, S., Shizuri, Y.; Yamamura, S.; Kawai, K.; Niwa, M.; Furukawa, H. *Heterocycles* **1991**, *32*, 307-310.

(12) (a) Cane, D. E.; Yang, C.-C. *J. Am. Chem. Soc.* **1987**, *109*, 1255-1257. (b) Cane, D. E.; Prabhakaran, P. C.; Tan, W.; Ott, W. R. *Tetrahedron Lett.* **1991**, *32*, 5457-5460.

(13) Yue, S.; Duncan, J. S.; Yamamoto, Y.; Hutchinson, C. R. *J. Am. Chem. Soc.* **1987**, *109*, 1253-1255.

(14) Cane, D. E.; Ott, W. R. *J. Am. Chem. Soc.* **1988**, *110*, 4840-4841.

(15) Spavold, Z. M.; Robinson, J. A. *J. Chem. Soc., Chem. Commun.* **1988**, 4-6.

(16) Huber, M. L. B.; Paschal, J. W.; Leeds, , J. P.; Kirst, H. A.; Wind, J. A.; Miller, F. D.; Turner, J. R. *Antimicrob. Agents and Chemother.* **1990**, *34*, 1535-1541.

(17) (a) Kinoshita, K.; Takenaka, S.; Hayashi, M. *J. Chem. Soc., Chem. Commun.* **1988**, 943-945. (b) Kinoshita, K.; Takenaka, S.; Hayashi, M. *J. Chem. Soc., Perkin Trans. 1* **1991**, 943-945.

(18) Takano, S.; Sekiguchi, Y.; Shimazaki, Y.; Ogasawara, K. *Tetrahedron Lett.* **1989**, *30*, 4001-4002.

(19) Suzuki, H.; Takenaka, S.; Kinoshita, K.; Morohoshi, T. *J. Antibiot.* **1990**, *43*, 1508-1511.

(20) Weber, J. M.; Leung, J. O.; Swanson, S. J.; Idler, K. B.; McAlpine, J. B. *Science* **1991**, *252*, 114-117.

(21) Donadio, S.; Staver, M. J.; McAlpine, J. B.; Swanson, S. J.; Katz, L. *Science* **1991**, *252*, 675-679.

(22) Cortes, J.; Haydock, S. F.; Roberts, G. A.; Bevitt, D. J.; Leadley, P. F. *Nature* **1990**, *348*, 176 -178.

(23) Hopwood, D. A.; Sherman, D. H. *Annu. Rev. Genet.* **1990**, *24*, 37-66.

(24) Caffrey, P.; Green, B.; Packman, L. C.; Rawlings, B. J.; Staunton, J.; Leadley, P. F. *Eur. J. Biochem.* **1991**, *195*, 823-830.

(25) Sherman, D. H.; Bibb, M. J.; Simpson, T. J.; Johnson, D.; Malpartida, F.; Fernandez-Moreno, M.; Martinez, E.; Hutchinson, C. R.; Hopwood, D. A. *Tetrahedron* **1991**, *31*, 6029-6043.

(26) Floss, H. G.; Strohl, W. R. *Tetrahedron* **1991**, *47*, 6045-6058.

(27) Kakinuma, S.; Ikeda, H.; Omura, S. *Tetrahedron* **1991**, *47*, 6059-6068.

(28) Strohl, W. R.; Bartel, P. L.; Li, Y.; Connors, N. C.; Woodman, R. H. *J. Industr. Microbiol.* **1991**, *7*, 163-174.

(29) Yoshizawa, Y.; Li, Z.; Reese, P. B.; Vederas, J. C. *J. Am. Chem. Soc.* **1990**, *112*, 3212-3213.

(30) For reviews, see: (a) Omura, S.; Tanaka, H. In *Macrolide Antibiotics, Chemistry, Biology, and Practice*; Omura, S., Ed.; Academic Press: Orlando, Florida, 1984; pp 351-404. (b) Schaffner, C. P. In *Macrolide Antibiotics, Chemistry, Biology, and Practice*; Omura, S., Ed.; Academic Press: Orlando, Florida, 1984; pp 457-507. (c) Kotler-Brajtburg, J.; Medoff, G., Kobayashi, G. S.; Boggs, S.; Schlessinger, D.; Pandey, R. C.; Rinehart, K. L., Jr. *Antimicrob. Agents Chemother.* **1979**, *15*, 716-722.

(31) (a) Pandey, R. C.; Guenther, E. C.; Aszalos, A. A.; Brajtburg, J. *J. Antibiot.* **1982**, *35*, 988-996. (b) Tytell, A. A.; McCarthy, F. J.; Fisher, W. P.; Bolhofer, W. A.; Charney, J. *Antibiot. Annu.* **1955**, 716-718. (c) Shirling, E. B.; Gottlieb, D. *Int. J. Syst. Bacteriol.* **1968**, *18*, 279-392. (d) Berry, M. P.; Whiting, M. C. *J. Chem. Soc.* **1964**, 862-863.

(32) Hazen, E. L.; Brown, R. *Science* **1950**, *112*, 423.

(33) Oroshnik, W.; Vining, L. C.; Mebane, A. D.; Taber, W. A. *Science* **1955**, *121*, 147-149.

(34) Vining, L. C. *Hindustan Antibiot. Bull.* **1960**, *3*, 37-54.

(35) Oroshnik, W.; Mebane, A. D. *Fortschr. Chem. Org. Naturst.* **1963**, *21*, 17-79.

(36) Hamilton-Miller, J. M. T. *Bacteriol. Rev.* **1973**, *37*, 166-196.

(37) Keller-Schierlein, V. W. *Fortschr. Chem. Org. Naturst.*. **1973**, *30*, 313-460.

(38) (a) Hammond, S. M. *Prog. Med. Chem.* **1977**, *14*, 105-179. (b) Tereshin, I. M. *Polyene Antibiotics-Present and Future*; Univ. Tokyo Press: Tokyo, Japan, 1976.

(39) (a) Medoff, G.; Vleriote, F.; Little, J. R.; Brajtburg, J.; Shore, V.; Presant, C. In *Augmenting Agents in Cancer Therapy*; Hersh, E. M., Ed.; Raven Press: New York, 1981; pp 479-495. (b) Bolard, J. *Biochem. Biophys. Acta* **1986**, *864*, 257-304.

(40) (a) Mechlinski, W.; Shaffner, C. P.; Ganis, P.; Avitabile, G. *Tetrahedron Lett.* **1970**, 3873-3876. (b) Ganis, P.; Avitabile, G.; Mechlinski, W.; Shaffner, C. P. *J. Am. Chem. Soc.* **1971**, *93*, 4560-4564.

(41) (a) Nicolaou, K. C.; Daines, R. A.; Ogawa, Y.; Chakraborty, T. K. *J. Am. Chem. Soc.* **1988**, *110*, 4696-4705. (b) Nicolaou, K. C.; Daines, R. A.; Uenishi, J.; Li, W. S.; Chakraborty, T. K. *J. Am. Chem.Soc.* **1988**, *110*, 4672-4685.

(42) Driver, M. J.; MacLachlan, W. S.; MacPherson, D. T.; Readshaw, S. A. *J. Chem. Soc., Chem. Commun.* **1990**, 636-638.

(43) Hanessian, S.; Botta, M. *Tetrahedron Lett.* **1987**, *28*, 1151-1154.

(44) Kennedy, R. M.; Abiko, A.; Masamune, S. *Tetrahedron Lett.* **1988**, *29*, 447-450.

(45) Oppong, I.; Pauls, H. W.; Liang, D.; Fraser-Reid, B. *J. Chem. Soc., Chem. Commun.* **1986**, 1241-1244.

(46) Nair, M. G.; Putnam, A. R.; Mishra, S. K.; Mulks, M. H.; Taft, W. H.; Keller, J. E.; Miller, J. R.; Zhu, P.-P.; Meinhart, J. D.; Lynn, D. G. *J. Nat. Prod.* **1989**, *52*, 797-809.

(47) (a) Sowinski, P.; Gariboldi, P.; Czerwinski, A. *J. Antibiot.* **1989**, *42*, 1631-1638. (b) Sowinski, P.; Gariboldi, P.; Pawlak, J. K.; Borowski, E. *J. Antibiot.* **1989**, *42*, 1639-1642.

(48) (a) Lancelin, J.-M.; Paquet, F.; Beau, J.-M. *Tetrahedron Lett.* **1988**, *29*, 2827-2830. (b) Prandi, J.; Beau, J.-M. *Tetrahedron Lett.* **1989**, *30*, 4517-4520. (c)

Lancelin, J.-M.; Beau, J.-M. *Tetrahedron Lett.* **1989**, *30*, 4521-4524. (d) Lancelin, J.-M.; Beau, J.-M. *J. Am. Chem. Soc.* **1990**, *112*, 4060-4061.

(49) Schreiber, S. L.; Goulet, M. T. *J. Am. Chem. Soc.* **1987**, *109*, 8120-8122.

(50) Oishi, T. *Pure Appl. Chem.* **1989**, *61*, 427-430.

(51) For reviews, see: (a) Martin, J.-F. *Annu. Rev. Microbiol.* **1977**, *31*, 13-38. (b) Martin, J.-F. In *Biochemstry and Genetic Regulation of Commercially Important Antibiotics*; Vining, L. C., Ed.; Addison-Wesley: Reading, 1983; pp 207-229. (c) Martin, J.-F. In *Macrolide Antibiotics, Chemistry, Biology, and Practice*; Omura, S., Ed.; Academic Press: Orlando, Florida, 1984; pp 405-424.

(52) Birch, A. J.; Holzapfel, C. W.; Rickards, R. W.; Djerassi, C.; Suzuki, M.; Westley, J.; Dutcher, J. D.; Thomas, R. *Tetrahedron Lett.* **1964**, 1485-1490.

(53) Perlman, D.; Semar, J. B. *Biotechnol. Bioeng.* **1965**, *7*, 133-137.

(54) Manwaring, D. G.; Rickards, R. W.; Gaudiano, G.; Nicolella, V. *J. Antibiot.* **1969**, *22*, 545-550.

(55) (a) Liu, C. M.; McDaniel, L. E.; Schaffner, C. P. *J. Antibiot.* **1972**, *25*, 116-121. (b) Martin, J.-F.; Liras, P. *J. Antibiot.* **1976**, *29*, 1306-1309.

(56) Liu, C. M.; McDaniel, L. E.; Schaffner, C. P. *J. Antibiot.* **1972**, *25*, 187-188.

(57) Belousova, I. I.; Lishnevskaya, E. B.; Elgart, R. E. *Antibiotiki (Moscow)* **1971**, *16*, 684-687.

(58) (a) Dhar, M. L.; Thaller, V.; Whiting, M. C. *Proc. Chem. Soc. (London)* **1960**, 310-311. (b) Cope, A. C.; Bly, R. K.; Burrows, E. P.; Ceder, O. J.; Ciganek, E.; Gilles, B. T.; Porter, R. F.; Johnson, H. E. *J. Am. Chem. Soc.* **1962**, *84*, 2170-2178.

(59) (a) Ammann, A.; Gottlieb, D.; Brock, T. D.; Carter, H. E.; Whitfield, G. B. *Phytopathology* **1955**, *45*, 559-563. (b) Whitfield, G. B.; Brock, T. D.; Ammann, A.; Gottlieb, D.; Carter, H. E. *J. Am. Chem. Soc.* **1955**, *77*, 4799-4801.

(60) (a) Noguchi, H.; Harrison, P. H.; Arai, K.; Nakashima, T. T.; Trimble, L. A.; Vederas, J. C. *J. Am. Chem. Soc.* **1988**, *110*, 2938-2945. (b) Harrison, P. H. *Studies on the Biosynthesis and Mechanism of Action of Polyene Antibiotics*; Ph. D. Thesis, University of Alberta, 1987.

(61) (a) Munro, H, D.; Musgrave, O. C.; Templeton, R. *J. Chem. Soc. C* **1967**, 947-948. (b) Starratt, A. N.; White, G. A. *Phytochemistry* **1968**, *7*, 1883-1884. (c) Grove, J. F. *J. Chem. Soc. C* **1971**, 2261-2263. (d) Hyeon, S.-B.; Ozaki, A.; Suzuki, A.; Tamura, S. *Agric. Biol. Chem.* **1976**, *40*, 1663-1664. (e) Caputo, O.; Viola, F. *Planta Med.* **1977**, *31*, 31-32. (f) Assante, G., Locci, R.; Camarda, L.; Merlini, L.; Nasini, G. *Phytochemistry* **1977**, *16*, 243-247. (g) Robeson, D. J.; Strobel, G. A. *Z. Naturforsch., C: Biosci.* **1981**, *36C*, 1081-1083. (h) Turner, W. B.; Aldridge, D. C. *Fungal Metabolites II*; Acdamic: New York, 1983; pp 167, 516. (i) Robeson, D. J.; Strobel, G. A.; Strange, R. N. *J. Nat. Prod.* **1985**, *48*, 139-141. (j) Kobayashi, A.; Yata, S.; Hino, T.; Kawazu, K. *Agric. Biol. Chem.* **1987**, *51*, 2857-2860.

(62) (a) Harvan, D. J.; Pero, R. W. In *Mycotoxins and Other Fungal Related Food Problems, Advances in Chemistry Series 149*; Rodricks, J. V., Ed.; American Chemistry Society: Washington, DC, 1976; pp 344-355. (b) Steele, J. A.; Uchytil, T. F.; Durbin, R. D.; Bhatnagar, P.; Rich, D. H. *Proc. Natl. Acad. Sci. USA* **1976**, *73*, 2245-2248.

(63) (a) Hansford, C. G. *Proc. Linn. Soc. Lond.* **1943**, *1*, 34-67. (b) Sciumbato, G. L.; Pinckard, J. A. *Plant Dis. Reptr.* **1974**, *58*, 201-202. (c) Balasubramanian, R.; Bhama, K. S. *Curr. Sci.* **1977**, *46*, 426. (d) Hadas, S.; Jakoby, T. *Phytoparasitica* **1981**, *9*, 252.

(64) Walker, H. L.; Sciumbato, G. L. *Plant Sci. Lett.* **1981**, *22*, 71-75.

(65) Kobayashi, A.; Hino, T.; Yata, S.; Itoh, T. J.; Sato, H.; Kawazu, K. *Agric. Biol. Chem.* **1988**, *52*, 3119-3123.

(66) Musgrave, O. C. *J. Chem. Soc.* **1956**, 4301-4305.

(67) Wasserman, H. H.; Gambale, R. J.; Pulwer, M. J. *Tetrahedron* **1981**, *37*, 4059-4067.

(68) Takahashi, T.; Ikeda, H.; Tsuji, J. *Tetrahedron Lett.* **1980**, *21*, 3885-3888.

(69) Gerlach, H. *Helv. Chim. Acta* **1977**, *60*, 3039-3044.

(70) Baker, P. M.; Bycroft, B. W.; Roberts, J. C. *J. Chem. Soc. C* **1967**, 1913-1915.

(71) Birch, A. J.; Musgrave, O. C.; Rickards, R. W.; Smith, H. *J. Chem. Soc.* **1959**, 3146-3152.

(72) Vesonder, R. F.; Stodola, F. H.; Wickerham, L. J.; Ellis, J. J.; Rohwedder, W. K. *Can. J. Chem.* **1971**, *49*, 2029-2032. For the total synthesis of recifeiolide, see: (a) Gerlach, H.; Oertle, K.; Thalmann, A. *Helv. Chim. Acta* **1976**, *59*, 755-760. (b) Corey, E. J.; Ulrich, P.; Fitzpatrick, J. M. *J. Am. Chem. Soc.* **1976**, *98*, 222-224.

(73) Hirota, A.; Isogai, A.; Sakai, H. *Agric. Biol. Chem.* **1981**, *45*, 799-800.

(74) Arai, K.; Rawlings, B. J.; Yoshizawa, Y.; Vederas, J. C. *J. Am. Chem. Soc.* **1989**, *111*, 3391-3399.

(75) Leete, E. *J. Nat. Prod.* **1982**, *45*, 197-205.

(76) Hedges, S. H.; Herbert, R. B.; Wormwald, P. C. *J. Chem. Soc., Chem. Commun.* **1983**, 145-147.

(77) McCarthy, F. J.; Fisher, W. P.; Charney, J.; Tytell, A. A. *Antibiot. Annu.* **1954-1955**, 719-723.

(78) Kindl, H. In *Fatty Acid Metabolism and Its Regulation*; Numa, A., Ed.; Elsevier: Amsterdam, 1984; pp181-204 and references therein.

(79) Stumpf, P. K. *Annu. Rev. Biochem.* **1969**, *38*, 159-212.

(80) Kang, S.-K.; Kim, W.-S.; Moon, B.-H. *Synthesis* **1985**, *12*, 1161-1162.

(81) Hendry, L. B.; Korzeniowski, S. H.; Hindenlang, D. M.; Kosarych, Z.; Mumma, R. O.; Jugovich, J. *J. Chem. Ecol.* **1975**, *1*, 317-322.

(82) Casamor, J. M.; Coll, J.; Guerrero, A.; Riba, M.; *Org. Prep. Proced. Int.* **1983**, *15*, 63-70.

(83) Degering, E. F.; Boatright, L. G. *J. Am. Chem. Soc.* **1950**, *72*, 5137-5139.

(84) Carlsen, P. H. J.; Katsuki, T; Martin, V. S.; Sharpless, K. B. *J. Org. Chem.* **1981**, *46*, 3936-3938.

(85) Boissonnas, R. A.; Guttmann, S.; Jaquenoud, P.-A.; Waller, J.-P. *Helv. Chim. Acta* **1955**, *38*, 1491-1501.

(86) Crombie, L.; Holloway, S. J. *J. Chem. Soc., Perkin 1* **1985**, 2425-2434.

(87) Anderson, R. J.; Henrick, C. A. *J. Am. Chem. Soc.* **1975**, *97*, 4327-4334.

(88) (a) Pandey, R. C.; Narasimhachari, N.; Rinehart, K. L., Jr.; Millington, D. S. *J. Am. Chem. Soc.* **1972**, *94*, 4306-4310. (b) Gopalkrishnan, K. S.; Narasimhachari, N.; Joshi, V. B.; Thirumalachar, M. J. *Nature* **1968**, *218*, 597-598.

(89) Doering, T. L.; Raper, J.; Buxbaum, L. U.; Adams, S. P.; Gordon, J.; Hart, G. W.; Englund, P. T. *Science* **1991**, *252*, 1851-1854.

(90) Skrede, S.; Narce, M.; Bergseth, S.; Bremer, J. *Biochim. Biophys. Acta* **1989**, *1005*, 296-302.

(91) Okazaki, H.; Ono, H.; Yamada, K.; Beppu, T.; Arima, K. *Agric. Biol. Chem.* **1973**, *37*, 2319-2325.

(92) Corey, E. J.; Venkateswarlu, A. *J. Am. Chem. Soc.* **1972**, *94*, 6190-6191.

(93) Kendall, P. M.; Johnson, Cook, C.-E. *J. Org. Chem.* **1979**, *44*, 1421-1424.

(94) Ceder, O.; Ryhage, R. *Acta Chem. Scand.* **1964**, *18*, 558-560.

(95) Bergy, M. E.; Eble, T. E. *Biochemistry* **1968**, *7*, 653-658.

(96) Pandey, R. C.; Rinehart, K. L. *J. Antibiot.* **1970**, *23*, 414-417.

(97) Edwards, D. M. *J. Antibiot.* **1989**, *42*, 322-324.

(98) Martin, J. R.; Rosenbrook, W. *Biochemistry* **1967**, *6*, 435-440.

(99) Ghisalba, O.; Traxler, P.; Nuesch, J. *J. Antibiot.* **1978**, *31*, 1124-1131.

(100) Evans, R.; Hanson, J. R.; Marten, T. *J. Chem. Soc., Perkin Trans 1* **1976**, 1212-1214.

(101) For reviews see: (a) *Cytochrome p-450: Structure, Mechanism and Biochemistry*; Ortiz de Montellano, P. R., ed.; Plenum: New York, 1986. (b) Akhtar, M.; Wright, J. N. *Nat. Prod. Rep.* **1991**, *8*, 527-551.

(102) Goolbaugh, R. C.; Hirano, S. S.; West, C. A. *Plant Physiol.* **1978**, *62*, 571-576.

(103) Izumi, K.; Kamiya, Y.; Sakurai, A.; Oshio, H.; Takahashi, N. *Plant Cell Physiol* **1985**, *26*, 821-827.

(104) Wauwe, V. J. P.; Janssen, P. A. J. *J. Med. Chem.* **1989**, *32*, 2232-2239.

(105) Oikawa, H.; Ichihara, A.; Sakamura, S. *J. Chem. Soc., Chem. Commun.* **1988**, 600-602.

(106) Oikawa, H.; Ichihara, A.; Sakamura, S. *J. Chem. Soc., Chem. Commun.* **1990**, 908-909.

(107) Townsend, C. A.; Christensen, S. B.; Davis, S. G. *J. Chem. Soc., Perkin Trans 1* **1988**, 839-861.

(108) (a) Ropp, G. A. *J. Am. Chem. Soc.* **1950**, *72*, 2299. (b) Reynolds, K. A.; O'Hagen, D.; Gani, D.; Robinson, J. A. *J. Chem. Soc., Perkin Trans. 1* **1988**, 3195-3207.

(109) Block, M. H.; Cane, D. E. *J. Org. Chem.* **1988**, *53*, 4923-4928.

(110) Martin, F. M. *Biosynthetic Studies on Pseudomonic Acid*; Ph.D. Thesis, University of Edinburgh, 1989.

(111) Hungerbuhler, E.; Seebach, D.; Wasmuth, D. *Helv. Chim. Acta* **1981**, *64*, 1467-1487.

(112) Mori, K. *Tetrahedron* **1981**, *37*, 1341-1342.

(113) (a) Gerstein, J.; Jencks, W. P. *J. Am. Chem. Soc.* **1964**, *86*, 4655-4663. (b) Schwab, J. M.; Klassen, J. B. *J. Am. Chem. Soc.* **1984**, *106*, 7217-7227.

(114) Yamada, S.-I.; Yokoyama, Y.; Shioiri, T. *J. Org. Chem.* **1974**, *39*, 3302-3303.

(115) Kelly, D. R.; Roberts, S.M. *Synth. Commun.* **1979**, *9*, 295-299.

(116) In *Protective Groups in Organic Synthesis*; Greene, T. W., Ed.; John Wiley & Sons: New York, 1981; pp21-22.

(117) Mancuso, A. J.; Huang, S.-L.; Swern, D. *J. Org. Chem.* **1978**, *43*, 2480-2482. For recent reviews on Swern oxidation and related reactions see ref.126.

(118) Tidwell, T. T. *Synthesis* **1990**, 857-870.

(119) Isler, O.; Gutmann, H.; Montavon, M.; Ruegg, R.; Ryser, G.; Zeller, P. *Helv. Chim. Acta* **1957**, *40*, 1243-1249.

(120) Parker, D. *J. Chem. Soc., Perkin Trans. 2* **1983**, 83-88.

(121) Corey, E. J.; Ponder, J. W.; Ulrich, P. *Tetrahedron Lett.* **1980**, *21*, 137-140.

(122) (a) De Koning, H.; Subramanian-Erhart, K. E. C.; Huisman, H. O. *Synth. Commun.* **1973**, *3*, 25-28. (b) Makin, S. M.; Telegina, N. I. *J. Gen. Chem. USSR* **1962**, *32*, 1982-1087. (c) Yanovskaya, L. A.; Rudenko, B. A.; Kucherov, V. F.; Stepanova, R. N.; Kogan, G. A. *Bull. Acad. Sci. USSR, Div. Chem. Sci.* **1962**, 2093-2099. (d) Gallagher, G., Jr.; Webb, R. L. *Synthesis* **1974**, 122-124.

(123) Buchta, E.; Andree, F. *Chem. Ber.* **1960**, *93*, 1349-1353.

(124) Mangera, O.; Rosenkranz, G.; Sondheimer, F.; *J. Chem. Soc.* **1953**, 2189-2191.

(125) Ungnade, H. E.; Hopkins, T. R. *J. Am. Chem. Soc* **1951**, *73*, 3091-3093.

(126) Heilbron, I.; Jones, E. R. H.; O'Sullivan, D. G. *J. Chem. Soc.* **1946**, 866-869.

(127) For discussions of mechanism of polyketide formation, see: Moore, R. N.; Bigam, G.; Chan, J. K.; Hogg, A. M.; Nakashima, T. T.; Vederas, J. C. *J. Am Chem. Soc.* **1985**, *107*, 3694-3701. Also see: Ref. 31, 47, 57, and 58.

(128) Townsend, C. A.; Christensen, S. B.; Trautwein, K. *J. Am. Chem. Soc.* **1984**, *106* , 3868-3869 and reference therein.

(129) For examples, see: (a) Birch, A. J. *Proc. Chem. Soc.* **1962**, 3-13. (b) Birkinshaw, J. H.; Gowlland, A. *Biochem. J.* **1962**, *84*, 342-347. (c) Grove, J. F. *J. Chem. Soc. (C)* **1970**, 1860-1863. (d) Cross, B. E.; Hendley, P. *J. Chem. Soc., Chem. Commun.* **1975**, 124-125. (e) Vederas, J. C.; Graf, W.; David, L.; Tamm, Ch. *Helv. Chim. Acta* **1975**, *58*, 1886-1898.

(130) Schulz, H. In *Biochemistry of Lipids and Membranes*; Vance, D. E., Vance, J. E., Eds.; Benjamin/Cummings: Menlo Park, CA, 1985; pp 116-142.

(131) For reviews, see: (a) Schulz, H. *Biochim. Biophys. Acta*, **1991**, *1081*, 109-120. (b) Bremer, J.; Osmundsen, H. In *Fatty Acid Metabolism and Its Regulation*; Numa, S., Ed; Elsevier: New York, 1984; pp 113-154.

(132) Lazarow, P. B.; de Duve, C. *Proc. Natl. Acad. Sci. USA* **1976**, *73*, 2043-2046.

(133) Roe, C. R.; Coates, P. M. In *The Metabolic Basis of Inherited Disease*; Scriver, C. R., Beaudet, A. C., Sly, W. S., Valle, D., Eds.; MaGraw-Hill: New York, 1989; 6th Edn., Vol I, pp 809-914.

(134) Lazaraw, P. B. and Moser, H. W. In *The Metabolic Basis of Inherited Disease*; Scriver, C. R., Beaudet, A. C., Sly, W. S., Valle, D., Eds.; MaGraw-Hill: New York, 1989; 6th Edn., Vol II., pp 1479-1510.

(135) Engel, A. G.; Angelini, C. *Science* **1973**, *179*, 899-902.

(136) DiMauro, S.; DiMauro, P. M. M. *Science* **1973**, *182*, 929-931.

(137) Hale, D. E.; Thorpe, C.; Braat, K.; Wright, J. H.; Roe, C. R.; Coaks, P. M.; Hashimoto, T.; Glasgow, A. M. In *Fatty Acid Oxidation: Clinical, Biochemical and Molecular Aspects*; Tanaka, K., Coates, P.M., eds.; Alan R. Liss: New York, 1990; pp 503-510.

(138) Roe, C. R.; Millington, D. S.; Norwood, D. L.; Kodo, W.; Sprecher, H.; Mohammed, B. S.; Nada, M.; Shultz, H.; McVie, R. *J. Clin. Invest.* **1990**, *85*, 1703-1707.

(139) Stremmel, W.; Strohmeyer, G.; Borchard, F.; Kochwa, S; Berk, P. D. *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 4-8.

(140) Sorrentino, D.; Stump, D.; Potter, B. J.; Robinson, R. B.; White, R.; Kiang, C.-L.; Berk, P. D. *J. Clin. Invest.* **1988**, *82*, 928-935.

(141) Stremmel, W.; Strohmeyer, G.; Berk, P. D. *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 3584-3588.

(142) Stremmel, W. *J. Biol. Chem.* **1987**, *262*, 6284-6289.

(143) Noy, N.; Donelly, T. M.; Zakim, D. *Biochemistry* **1986**, *25*, 2013-2021.

(144) Cooper, R.; Noy, N.; Zakim, D. *Biochemistry* **1987**, *26*, 5890-5896.

(145) Furuta, S.; Miyazawa, S.; Hashimoto, T. *J. Biochem.* **1981**, *90*, 1739-1750.

(146) Ikeda, Y.; Okamura-Ikeda, K.; Manaka, K. *J. Biol. Chem.* **1985**, *260*, 1311-1325.

(147) Davidson, B.; Schulz, H. *Arch. Biochem. Biophys.* **1982**, *213*, 155-162.

(148) Dommes, V.; Kunau, W.-H. *J. Biol. Chem.* **1984**, *259*, 1789-1797.

(149) Kelly, D. P.; Kim, J.-J.; Billadello, J. J.; Hainline, B. E.; Chu, T. W.; Strauss, A. W. *Proc. Natl. Acad. Sci. USA* **1987**, *84*, 4068-4072.

(150) Matsubara, Y.; Kraus, J. P.; Ozasa, H.; Glassberg, R.; Finocchiaro, G.; Ikeda, Y.; Mole, J.; Rosenberg, L. E.; Tanaka, K. *J. Biol. Chem.* **1987**, *262*, 10104-10108.

(151) Matsubara, Y.; Indo, Y.; Naito, E.; Ozasa, H.; Glassberg, R.; Vockley, J.; Ikeda, Y.; Kraus, J.; Tanaka, K. *J. Biol. Chem.* **1989**, *264*, 16321-16331.

(152) Naito, E.; Ozasa, H.; Ikeda, Y.; Tanaka, K. *J. Clin. Invest.* **1989**, *83*, 1605-1613.

(153) Kim, J.-J. P.; Wu, J. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 6677-6681.

(154) Frerman, F. E. *Biochem. Soc. Trans.* **1988**, *16*, 416-418 and references therein.

(155) Frerman, F. E.; Turnbull, D. In *Fatty Acid Oxidation: Clinical, Biochemical and Molecular Aspects*; Tanaka, K., Coates, P. M., eds.; Alan R. Liss: New York 1990; pp 79-89.

(156) Thorpe, C. In *Fatty Acid Oxidation: Clinical, Biochemical and Molecular Aspects*; Tanaka, K.; Coates, P. M., eds.; Alan R. Liss: New York 1990; pp 91-105.

(157) Schopfer, L.; Massey, V.; Ghisla, S.; Thorpe, C. *Biochemistry* **1988**, *27*, 6599-6611.

(158) Pohl, B.; Raichle, T.; Ghisla, S. *Eur. J. Biochem.* **1986**, *160*, 109-115.

(159) Frerman, F. E.; Miziorko, H. M.; Beckmann, J. D. *J. Biol. Chem.* **1980**, *255*, 11192-11198.

(160) Powell, P. J.; Lau, S.-M.; Killian, D.; Thorpe, C. *Biochemistry* **1987**, *26*, 3704-3711.

(161) Biellmann, J. F.; Hirth, C. G. *FEBS Lett.* **1970**, *9*, 335-336.

(162) Ghisla, S.; Thorpe, C.; Massey, V. *Biochemstry* **1984**, *23*, 3154-3161.

(163) Ikeda, Y.; Hine, D. G.; Okamura-Ikeda, Tanaka, K. *J. Biol. Chem.* **1985**, *260*, 1326-1337.

(164) Wenz, A.; Thorpe, C.; Ghisla, S. *J. Biol. Chem.* **1981**, *256*, 9809-9812.

(165) Wenz, A.; Ghisla, S.; Thorpe, C. *Eur. J. Biochem.* **1985**, *147*, 553-560.

(166) Fendrich, G.; Abeles, R. H. *Biochemistry* **1982**, *21*, 6685-5594.

(167) Thorpe, C.; Ciardelli, T. L.; Stewart, C. J.; Wieland, T. *Eur. J. Biochem.* **1981**, *118*, 279-282.

(168) Kunau, W.-H.; Bühne, S.; De La Garza, M.; Kionka, C.; Mateblowski, M.; Schultz-Borchard, U.; Thieringer, R. *Biochem. Soc. Trans.* **1988**, *16*, 418-420.

(169) For reviews, see: Osmundsen, H.; Hovik, R. *Biochem. Soc. Trans.* **1988**, *16*, 420-422.

(170) Engeland, K.; Kindl, H. *Eur. J. Chem.* **1991**, *196*, 699-705.

(171) *Hypoglycin* ; Kean, E. A., ed.; Academic Press: New York, 1975.

(172) Hill, K. R. *West Indian Med. J.* **1952**, *1*, 243-364.

(173) Stuart, K. L. In *Hypoglycin*; Kean, E. A., Ed.; Academic: New York, 1975; pp 39-44.

(174) Tanaka, K. In *Handbook of Clinical Neurology*; Vinken, P. J., Bruyn, G.W., Eds.; Elsevier/North Holland: Amsterdam, 1979; Vol.37(II), pp 511-539.

(175) Ghisla, S.; Melde, K.; Zeller, H. D.; Boschert, W. In *Fatty Acid Oxidation: Clinical, Biochemical and Molecular Aspectts;* Tanaka, K., Coates, P. M., eds.; Alan R. Liss: New York,1990; pp 185-192.

(176) Schulz, H. In *Fatty Acid Oxidation: Clinical, Biochemical and Molecular Aspectts;* Tanaka, K., Coates, P. M., eds.; Alan R. Liss: New York,1990; pp 153-165.

(177) (a) Lai, M.-T.; Liu, H.-W. *J. Am. Chem. Soc.* **1990**, *112*, 4034-4035. (b) Lai, M.-T.; Liu, L.-D.; Liu, H.-W. *J. Am. Chem. Soc.* **1991**, *113*, 7388-7397.

(178) Lenn, N. D.; Shih, Y.; Stankovich, M. T.; Liu, H.-W. *J. Am. Chem. Soc.* **1989**, *111*, 3065-3067.

(179) Thorpe, C.; *TIBS* **1989**, *14*, 148-151.

(180) Holland, P. C.; Clark, M. G.; Bloxham, D. P.; *Biochemistry* **1973**, *12*, 3309-3315.

(181) Freund, K.; Mizzer, J.; Dick, W.; Thorpe, C. *Biochemistry* **1985**, *24*, 5996-6002.

(182) Yokoyama, Y.; Shioiri, T.; Yamada, S.-I. *Chem. Pharm. Bull.* **1977**, *25*, 2423-2429.

(183) Kruithof, K. J. H.; Schmitz, R.F.; Klumpp, G. W. *Tetrahedron* **1983**, *39*, 3073-3081.

(184) Takahata, H.; Takamatsu, T.; Yamazaki, T. *J. Org. Chem.* **1989**, *54*, 4812-4822.

(185) Green, G.; Griffith, W. P.; Hollinshead, D. M.; Ley, S. V.; Schröder, M. *J. Chem. Soc., Perkin Trans. I* **1984**, 681-686.

(186) Hovik, R.; Osmundsen, H.; Berge, R.; Aarsland, A.; Bergseth, S.; Bremer, J. *Biochem. J.* **1990**, *270*, 167-173.

(187) Spydevold, Ø.; Bremer, J. *Biochim. Biophys. Acta.* **1989**, *1003*, 72-79.

(188) (a) Billington, D.; Sherratt, H. S. A. *Methods Enzymol.* **1981**, *72*, 610-617. (b) Kean, E. A. *J. Pharm. Pharmac.* **1974**, *26*, 639-640.

(189) (a) Baldwin, J. E.; Parker, D. W. *J. Org. Chem.* **1987**, *52*, 1475-1477. (b) Kohn, H.; Bean, M. B.; von Rohrscheidt, C.; Willcott III, M. R.; Warnhoff, E. W. *Tetrahedron* **1981**, *37*, 3195-3201.

(190) Ogilvie, K. K; Iwacha, D. J. *Tetrahedron Lett.* **1973**, 317-319.

(191) Ernst, B.; Wagner, B. *Helv. Chim. Acta.* **1989**, *72*, 165-171.

(192) (a) House, H. O.; Jones, V. K.; Frank, G. A. *J. Org. Chem.* **1964**, *29*, 3327-3333. (b) Larock, R. C.; Narayanan, K. *J. Org. Chem.* **1984**, *49*, 3411-3413.

(193) Kirchner, G.; Scollar, M. P.; Klibanov, A. M. *J. Am. Chem. Soc.* **1985**, *107*, 7072-7076.

(194) Diakur, J.; Nakashima, T. T.; Vederas, J. C. *Can. J. Chem.* **1980**, *58*, 1311-1315.

(195) Fraser, R. R. In *Asymmetric Synthesis*; Morrison, J. D., ed.; Academic: New York, 1983; Vol, pp 183-196.

(196) Seebach, D.; Sutter, M. A.; Weber, R. H.; Züger, M. F. *Org. Synth.***1990**, *7*, 215-220.

(197) Sugai, T.; Ohta, H. *Agric. Biol. Chem.* **1989**, *53*, 2009-2010.

(198) *Purification of Laboratory Chemicals*; Perrin, D. D., Armarego, W. L. F., Perrin, D. R., eds.; Pergamon: New York, 1986; 2nd ed.

(199) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923-2925.

(200) Hase, J.; Ohura, H. *Chem. Pharm. Bull.* **1954**, *2*, 368-372.

(201) Thewalt, K.; Rudolph, W. *J. Prakt. Chem.* **1964**, *26*, 233-261.

(202) Chuit, P.; Hauser, J. *Helv. Chim. Acta* **1929**, *12*, 463-493.

(203) Kinoshita, M.; Umezawa, S. *Bull. Chem. Soc. Jpn.* **1961**, *34*, 308-312.

(204) Boyer, J. M. *Antimicrob. Agents Chemother.* **1976**, 9, 1070-1071.

(205) Elegant experiments on incorporation of advanced intermediates into aspyrone have been published after completion of this manuscript: Staunton, J.; Sutkowski, A. C. *J. Chem. Soc., Chem. Commun.* **1991**, 1108-1110 and accompanying papers.